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VARIATIONS IN INTEGUMENTARY GLAND ACTIVITY AND SCENT MARKING IN COLUMBIAN GROUND-SQUIRRELS (*SPERMOPHILUS C. COLUMBIANUS*)

by

© V. KEITH KIVETT

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Variations in Integumentary Gland Activity and Scent Marking in Columbian Ground Squirrels (*Spermophilus c. columbianus*)," submitted by V. Keith Kivett in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Columbian ground squirrels, *Spermophilus columbianus*, live in social groups in which olfaction seems to play an important role in maintaining group and territorial integrity. In this study I investigated seasonal variations in secretory activity of integumentary glands and scent marking behavior, emphasizing adult males.

Histological examination of suspected integumentary gland sites revealed apocrine-type secretory glands in the oral angle, the anal region, and a dorsal gland field composed of approximately 60 individual glands. Sebaceous glands were associated with hair follicles on the entire body but those near ducts from the underlying apocrine glands in the oral and dorsal regions were larger. Anal glands consisted of a combination of apocrine and sebaceous glands, not associated with hair follicles, located in retractable papillae lateral and ventral to the anus.

Secretory activity of apocrine glands in the oral and dorsal regions peaked during the breeding season in adult males and during the pregnancy-lactation period in adult females. Frequency of scent marking in males was maximal during the breeding season when territorial behavior was most vigorous. During this time adult males excluded other males from a territory that usually encompassed two or three adult females. Marking by adult females was observed infrequently although increases were noted during the pregnancy-lactation period when a single burrow in which their young are reared is defended.

Secretory activity of oral and dorsal glands increased significantly in adult males after testosterone-propionate was administered. In a controlled experiment, scent marking and aggression were higher after injections of this hormone. A significant positive correlation found between testes weight and marking frequency indicates that testosterone may influence behavior patterns as well as integumentary gland activity.

The dominant animal of pairs of males in captivity marked more frequently than subordinates. When a reversal in dominance occurred after the subordinate received testosterone injections, the former dominant male's marking decreased and the former subordinate's marking increased, both significantly.

Sharp increases in marking and aggression in adult males, based on field observations, coincided with emergence from hibernation of adult females. In a controlled experiment designed to investigate these possible correlations, the presence of a reproductively active female was accompanied by significantly higher levels of aggression between two rival males. Also, significant increases in marking were recorded for adult males in both the presence (separately) of another adult male and a female.

These data suggest that scent marking is closely associated with territorial behavior and accompanying aggression. In males, the presence of adult females (reproductively active) may elicit marking and territorial behavior, possibly because a male's reproductive fitness is enhanced by a female's presence within his territory. Marking and territoriality seem to have a maternal component of motivation in females.

Motivation and control of scent marking and the involvement of scent in the social structure of this species are discussed. Integration of scent with other communication modalities utilized by *S. columbianus* is also considered.

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INTRODUCTION

Vertebrate communication has evolved along several lines in order to meet, within limits, environmental and social demands that confront a species. The olfactory mode of communication, used by most mammals to some extent, has advantages and disadvantages depending on the situation in which it is employed (Mykytowycz 1972). As would be expected, olfaction is used extensively by nocturnal animals, but is also part of the signalling repertoire of gregarious and highly social diurnal animals which also use other modes of communication extensively. Highly social animals may require more communication modalities to maintain some degree of stability in complex social systems; thus, olfaction might have become an integral part of their communication system.

Recent studies of mammalian olfactory communication have revealed specialized integumentary glands in most mammalian orders (Muller-Schwarze 1967) and, in some instances, behavior patterns that appear to be associated with potential sites of scent production have been described. When these potential sites were examined histologically two main types of scent-producing glands were found, holocrine sebaceous and apocrine sudoriferous (Mykytowycz 1970).

Early investigators regarded scent marking as territorial, but as behavioral information accumulated other functions were suggested and the territorial function was de-emphasized somewhat. However, in recent reviews of scent marking both Ralls (1971) and Johnson (1973) state that the association of marking with aggression and dominance is suggestive of threat or territorial motivation.

Seasonal patterns of integumentary gland activity have been reported in some mammalian species (e.g., rabbits, Mykytowycz 1965, 1966a, b; water voles, Stoddart 1972; grey squirrels, Taylor 1968), but prior histological studies of the integumentary glands of the genus *Spermophilus* have been limited to description only (Hatt 1926, Sleggs 1926, Ouay 1965).

Researchers have described behavior patterns that suggest the presence of scent communication in several North American species of ground squirrels and have sometimes speculated on scent functions (e.g., *S. undulatus*, Watton and Keenleyside 1974; *S. armatus*, Balph and Stokes 1963; *S. beecheyi*, Linsdale 1946; etc.). Evidence that olfactory communication may be as vital as the more frequently studied visual and auditory signalling systems has been shown in an extensive behavioral study of the Columbian ground squirrels by Steiner (1970a, b, 1973, 1974). He described several scent-related behavior patterns in this animal's complex social system and emphasized the likely importance of olfaction in this system.

The Columbian ground squirrels (*Spermophilus columbianus columbianus*) seemed particularly well suited for a study of olfactory communication in a complex social system because of the available information on the behavior of this species, their relative abundance, and their observability.

Because of the paucity of available information on the integumentary regions involved in scent production, my first objective was to locate the integument regions that contained glands with a potential for scent production and then to determine if these glands exhibited seasonal variations. Next, specific behaviors that involved

scent were examined and related to gland activity. Emphasis was placed on scent marking by the adult males and the role of scent in this species' social system. Along with field observations, experiments were conducted to relate behaviors that involved scent to social interactions.

The field portions of this study were conducted in the Canadian Life Zone (elev. 1500 m) on the Highwood River drainage. This area is located in the Rocky Mountain Foothills 46 kilometers west of Longview in southwestern Alberta (114° 39' W, 50° 23' N).

In this area *S. columbianus* lives in social groups several of which make up large continuous colonies in flat grassy areas and on gentle slopes that are mainly devoid of trees. On steeper slopes and in more heavily treed areas some groups are separated spatially by 25 to 75 meters. The structure of a social group was variable, both spatially and seasonally, but usually consisted of one adult male, two to three adult females, several yearlings, and young of the year.

Most of the animals that were taken for histological analysis and all general behavior and scent marking data were collected within a two-kilometer radius. This was done to make data comparable on a temporal basis and to ensure that only one population was sampled.

Field data for this study were collected during the spring and summer of 1972 (spring emergence, April 27, until hibernation, August 12), the spring of 1973 (spring emergence, April 18 to May 28), and for one week in the early spring of 1974 (April 12 to April 16).

Controlled experiments were conducted at The University of Alberta during the summers of 1973 and 1974.

STATISTICAL PROCEDURES

Data that were amenable to statistical analysis were treated with several different statistical tests as suggested by Sokal and Rohlf (1969).

Analysis of Variance and the "t" test were applied to gland characteristics that were quantified. When significant "F" values were found, Duncan's Multiple Range Test was used to determine which means differed significantly. Whenever correlations are presented, Pearson's Correlation Coefficients were used, unless otherwise stated. Prior to using parametric tests, data were checked for normality with the Kolmogorov-Smirnov Test for Normality.

Behavior data from the Scent Marking and Social Status and Scent Marking and Social Setting Experiments were treated with Analysis of Variance and Duncan's Multiple Range Test.

Chi Square was used to test for significance in the Differential Responses to Scent Stimuli Experiment. A 1:1:1 goodness of fit to Chi Square was used to compare responses to different scent and a 1:1 goodness of fit test to compare equivalent time intervals in breeding and postbreeding experiments. A 2 x 2 contingency table was used to compare total marking in the breeding and postbreeding periods.

An Olivetti desk programma and APL programs run on an IBM 360 computer were used. The five-percent probability level was selected as the rejection level for the null hypothesis.

ANNUAL CYCLE OF COLUMBIAN GROUND SQUIRRELS

Although the life history of Columbian ground squirrels was studied by Shaw (1924, 1925a-h, 1926a-c, and 1945) and by Manville (1959), neither author described in detail behavior patterns that occur in the early active season (emergence from hibernation through the breeding season). To establish a time base for seasonal variations in integumentary gland activity and scent marking, I describe here the annual cycle of Columbian ground squirrels, emphasizing early season behavior. The dates used apply to observations I made in 1972, 1973 and 1974, although there is no doubt some year-to-year variability. This description is based, primarily, on observations made on three adjacent groups and also contains observations from several other groups in the immediate area. Methods used are described in the Field Studies Section.

Adult male Columbian ground squirrels emerged from hibernation three to four days earlier than adult females. On the first day out of hibernation males remained near the burrow entrance from which they emerged, neither feeding nor interacting with males in adjacent areas (future territories).

During the subsequent three to four day period, general activity and aggression encounters between males increased gradually. Although most encounters involved only broadside displays (see Steiner 1970b) across territorial boundaries, a few chases and overt fights were observed. Scent marking, which involves rubbing of the oral angle regions or a twisting movement that results in the dorsal gland field contacting the substrate, occurred infrequently during this period (see Scent Marking section and Steiner 1970a, 1974).

On the day (April 20) that adult females emerged from hibernation within the burrow systems of the adult males, marked changes were noted in behavior of the adult males. Agonistic encounters took the form of aggressive male-male chases and frequent fights across vigorously defended territorial boundaries. Males inspected burrow entrances and marked prominent objects during regular movements throughout their territory. Steiner (1970a) referred to this behavior as burrow patrolling. Frequency of scent marking, primarily with the oral gland, increased five-fold over the preceding day. Greeting behavior among group members, which involves nasal investigation of the oral gland, was also high at this time (for details see Steiner 1970b). These patterns of behavior with their aggressive overtones continued over the next two weeks with the frequency of male-male encounters and scent marking gradually decreasing after the first week.

The adult females were submissive and tolerant of both males and other females for approximately two weeks after their emergence from hibernation. During the early part of this period breeding occurred. Most females were pregnant by May 1 and thereafter became less tolerant of other females, their group's dominant male, and neighboring males.

By May 4 the pregnant females had moved away from central to peripheral burrows, usually still within the same male's territory. A small territory (approximately five meters in diameter) which centered on a single burrow was defended against all intruders including other group members until after their young emerged (June 20). Females appeared to be more aggressive for three to four days after the first emergence of their young, but chases and occasional fights with other females and with males soon became less intense. A female's

aggressiveness toward the male whose territory encompassed her small territory was low in relation to her aggressiveness toward males in neighboring territories. Females chased neighboring males for long distances (15-20 m); however, if her group male trespassed on her territory the chase covered only one or two meters.

Scent marking by territorial females (both mouth corner rubbing and twist type) was observed during the pregnancy-lactation period, although much less frequently than in breeding males.

From mid-May until hibernation in mid-August adult males who held territories became more tolerant of territorial intrusions by neighboring males. Territorial behavior never abated completely, but it seemed that individual distance became more important in controlling aggressive behavior. For example, a male would not traverse a great distance to exclude an intruding male from his territory, but if the intruder moves within what appears to be some critical distance, the resident would initiate a chase (see also Balph and Stokes 1963, Barash 1973).

Throughout the breeding season adult males were tolerant of yearlings (first yearling observed on April 23), but from mid-May until early June the adult males were aggressive toward yearlings, especially toward yearling males. Some of the yearling males that were trapped in mid-May had pigmented scrotums and enlarged testes, indicative of some degree of reproductive development. The reproductive development and increased aggressiveness of the yearling males may contribute to their presence within a male's territory not being tolerated. Adult females also directed aggression toward the yearling squirrels when they entered their territories. Most yearling males and some yearling females disappeared from the groups in late May or June. The aggression directed toward

these animals may have contributed to their apparent dispersal.

The hibernation date of adult females approximates that of adult males, two to three weeks before the juveniles and yearlings.

FIELD STUDIES

SCENT MARKING AND RELATED BEHAVIOR

In the field portion of this study I attempted to record scent marking and other behavioral data that were scent related. In addition, animals used in the histological evaluation of the integumentary gland were collected.

Methods

To enhance visual recognition of animals involved in the study of frequency of scent marking and those collected for histological examination of suspected integumentary gland regions, I tagged both ears of each squirrel with a numbered tag (#1 monel fingerling tags), enclosing a colored plastic disk (12 mm diameter). For initial and subsequent captures National Live Traps (17 x 17 x 35 cm) (National Live Trap Co., Tomahawk, Wis.) were baited with peanut butter and were positioned at burrow entrances. The squirrels were restrained in a nylon mesh bag while being weighed (Pesola spring scale, 0-1500 g), sexed, and ear-tagged.

Before specific animals were collected for later histological analysis or before behavioral information was recorded, I determined the social status, when possible, of all group members. In many instances, with the exception of weight at time of capture, status position and general behavior of an animal were the only criteria I had to separate the yearling and adult age classes. This distinction was essential for

investigation of seasonal variations in gland activity as well as behavior observations.

Three to four observation periods of three hours each, usually beginning at morning emergence, were sufficient for me to evaluate status within one group. Observations were made with 10 x 50 binoculars from a concealed location or from a great enough distance so that behavior patterns were not influenced by the observer's presence. First, the residents of a burrow system were determined by observing morning emergence; then, all animals were categorized as to age class (based on behavior) and dominance status within each age class and within the entire group, emphasizing the adults. Dominance status was based, primarily, on the following behavior patterns: (1) the winner of aggressive encounters; (2) the initiator of greeting behavior; (3) the squirrel that displaced another from a feeding site.

To investigate seasonal variations in gland activity I sacrificed selected animals, primarily adults, at two-week intervals throughout the active season (1972). During the next two active seasons (1973 and 1974) animals were only collected at times of intense gland activity and at some gaps in the data from 1972. A few animals were collected from other populations but these are not included in seasonal comparisons. After ether asphyxiation the reproductive tracts and skins were removed and preserved in ethanol, formalin, and acetic acid (A.F.A.).

To facilitate quantification of oral marking, which is rather subtle, I used marking pegs, 3.7 cm cubes of acrylic (transparent and neutral in color) mounted on removable, sharpened stakes (12 cm in length). Besides allowing manipulation of the scent, the pegs provided an object on which a scent mark is readily detectable by man. Because this species

preferentially marks prominent objects (Steiner 1974), the pegs were readily marked. During the collection of marking data I positioned ten marking pegs on major burrow mounds (usually 8 to 10) in a group. The burrow mounds were selected because they provided an observable site that was frequently scent marked.

Prior to morning emergence of the animals, marking pegs were positioned and a vehicle was strategically parked for use as a blind. The number of times the pegs were marked by a group male was then recorded daily for the first two hours after the male emerged in the morning (total 86 days in two seasons both encompassing the breeding season), weather permitting. Scent marking, dominance relationships, location of territorial boundaries, and other related data were recorded, primarily, from two immediately adjacent groups.

The marking pegs were collected and scent marks verified by smelling them after the observation period. I then washed the pegs with soap and water and rinsed them in 70% ethanol to remove any scent that might affect marking behavior on subsequent days.

Since scent-related behavior and integumentary gland activity appeared to be closely associated with the animal's reproductive condition, the following categories, based on reproductive state, were used as convenient divisions of the active season. For adult males, the interval between the emergence from hibernation until two and one-half to three weeks later (breeding) was separated from the remainder of the active season (post-breeding). The end of the breeding season was approximated by backdating from the date the latest litter was first observed above ground, using twenty-four days as the length of the gestation period and four weeks as the interval after birth until the

young were able to leave the nest (after Soper 1964).

Adult females were classified as breeding, pregnant, lactating, or post-lactating based on the following criteria: (1) breeding--emergence from hibernation until pregnant (the earliest stage determined when embryos produced visible swelling in the uterus); (2) pregnant--embryos visible as swellings in the uterine horns; (3) lactating--distinct embryo scars and active mammary glands; (4) post-lactating--inactive mammary glands and indistinct embryo scars.

Some reproductive development was also noted in the yearling age class. Scrotal pigmentation and testis enlargement (approximately one-half the adult testes weight) in the males and a swelling of the vulva in some females was noted, but neither sex participated in breeding as yearlings.

Yearlings could be distinguished from adults on the basis of body weight for approximately the first two months of the active season, but as fall approached the yearling and adult weights were not separable. However, the two age classes could still be easily separated by behavioral differences. Yearlings engaged in play behavior frequently; play is seldom seen in adults.

Results

Scent marking. Columbian ground squirrels assume different postures when scent marking with integumentary glands located in the oral, dorsal, and anal regions. Marking with the oral gland (mouth corner rubbing) is accomplished by a side-tilting of the head until the oral angle region contacts the object being marked; a quick forward thrust of the head completes the mark (Fig. 1 and Steiner 1974). Twist marking

frequently begins with oral gland marking after which a twisting motion allows the dorsal gland field to contact the sugstrate as the animal rolls on to its back (See Steiner 1974 for details). During anal drag marking, described by Steiner (1974), squirrels flatten their ventral side against the substrate and drag themselves forward several inches. As discussed in the Possible Functions and Meanings of Scent Marks section, secretions form the anal gland may or may not contribute to the mark that is deposited.

As mentioned in the Annual Cycle section, scent marking by adult males was low during the three to four day period between their emergence from hibernation and the emergence from hibernation of the adult females (Fig. 2). A striking increase in marking with the oral gland coincided with emergence from hibernation of adult females. High levels of marking were maintained throughout the breeding season (Fig. 2). After this time, frequency of marking declined to a low level that was maintained until hibernation in mid-August.

Adult males in the groups that were involved in the scent marking study marked, primarily, with the oral gland. Twist marking also occurred more frequently during the breeding season but at such a low level that quantification did not reveal a pattern as found for oral marking.

Anal drag marking was recorded in the field on marking pegs three times, only during the breeding season. This type of marking was difficult to verify unless performed on a marking peg since this behavior resembles stretching during comfort behavior; thus, many instances were likely overlooked. Anal drag by the captive male that was used in the Differential Responses to Scent Stimuli experiment was, likewise,

restricted to the breeding season.

Scent marking and territorial behavior. Information on changes in territorial ownership and boundary location of male *S. columbianus* in a colonial situation were recorded in conjunction with collection of scent marking data (April 18-May 28, 1973).

Although territory boundaries were stable for the first few days after the males emerged from hibernation, this soon changed. On April 23 male GW displaced male RL from the south two burrows of his territory (Fig. 3a). At the time, male RL was holding a relatively large territory and appeared to be neglecting, in terms of burrow patrol, the portion that was lost. Only two brief fights preceded the boundary change, even though male RL had held this territory throughout the summer of 1972.

Also, on April 23, male RL's territory boundaries were challenged by males BL and WW. Intense fighting, especially between males RL and BL, typified these intrusions. In the subsequent five-day period male RL was decisively beaten by male BL in almost every encounter. Male RL's inability to defeat male BL was the probable cause of RL's territory being reduced to two burrows on May 5 (Fig. 3b) and then his exclusion from these burrows for two days (May 6 and 7) (Fig. 3c).

During this latter period (May 6 and 7) BL expanded his territory northward thereby displacing male YY to a less suitable location (sparsely vegetated and without females) north of the pond and also southward where he displaced male GW from two burrows (Fig. 3d).

Male RL was observed west of the pond on the periphery of an untagged male's territory on May 6 and 7. On May 8 RL regained, after a

brief fight with BL, the two burrows from which he was displaced on May 6 (Fig. 3e). BL seemed to be concentrating his territorial defense on male YY's former territory; this may have enabled male BY to take over the south two burrows of his territory (May 8) (Fig. 3e).

Despite daily fighting over territorial boundaries with RL, BL maintained his large territory. Apparently during one of these frequent battles, BL suffered an eye injury that worsened daily. As swelling reduced the vision in his right eye, BL became less aggressive and seemed weakened. RL now initiated more fights and trespassed further into BL's territory. Subsequently, a boundary change whereby RL recovered the major portion of his former territory was preceded by a vigorous fight that ended in a decisive victory for RL over BL. Territories remained stable until May 31 when observations were discontinued (Fig. 3f).

Throughout the changes in territory ownership by the males, the behavior of the adult females was similar to that observed in the same groups the previous year when the situation was relatively stable. The females (the number in each territory indicated on Fig. 3a) avoided the male fighting and remained on the territory where they had emerged from hibernation. After the breeding season they established small territories around single burrows (see Annual Cycle for details).

All of the males that held territories during the observation period had been tagged the previous year (1972). Male BL had been tagged on August 10, as an adult or possibly a large yearling (wt. 860 g) west of the pond. Male YY had been tagged in 1972 (retagged April 24, 1973) and was defending the same territory as the previous year. GW had also been tagged as an adult in 1972. As mentioned earlier, RL had held the same territory in 1972.

Frequency of marking changed rapidly during the early portion of the active season; therefore, marking values that were separated by more than one or two days were not comparable. Thus, more emphasis was placed on day-to-day changes in marking frequency that corresponded to shifts in territory boundaries.

The highest level of marking was recorded for male RL from April 21 through April 27 ($\bar{x}$ = 5 pegs/hr) during the peak of the breeding season prior to any major territory changes.

On May 5, after male RL's territory was reduced to two burrows (Fig. 3b) RL did not mark any pegs during the observation period. The preceding day (May 4) when his territory was still intact, male RL marked at a rate of two pegs per hour. It was impossible to record male RL's marking on May 6 and 7 because of his displacement to an unobservable area. But from May 8-16 as RL gradually regained his lost territory, his marking increased despite the usual decreases in marking that occurs during this time.

As male BL acquired a larger territory May 5-7 (Fig. 3b, c, d) his marking (both peg and ground) and burrow patrolling increased proportionally. By May 7 (Fig. 3d) BL was defending the largest territory observed that spring. After losing approximately one-half of his territory to male RL on May 16 (Fig. 3f) male BL marked the ground less frequently: although his peg marking rate was unchanged.

Discussion

Scent marking. In addition to this study, Mykytowycz (1965, 1966a, b) for rabbits, *Oryctolagus cuniculus*, Muller-Schwarze (1972) for black-tailed deer, *Odocoileus hemionus*, and others have reported peaks

in marking by males during the breeding season; however, data on cyclic variations in marking behavior of mammals are few (Johnson 1973).

Marking is not restricted entirely to the breeding season. In the grey squirrel, *Sciurus carolinensis*, Taylor (1968) found peaks of marking outside as well as within the breeding season. In this study, marking by male *S. columbianus* was reduced in the postbreeding season but never disappeared. If the motivation for marking by males has territorial implications then one would not expect a complete cessation because territorial behavior is maintained, although to a lesser degree, in the postbreeding period.

It is widely accepted that males mark more than females (Mykytowycz 1965, Thiessen, Blum and Lindzey 1970, Steiner 1970a), and this appears to be the case in Columbian ground squirrels. Females that were most aggressive in defense of the area surrounding their burrow in which their young were reared seemed to mark more frequently than others. Of four females that were observed regularly, two were aggressive and marked frequently. These two squirrels had reared litters the previous season at the same locations and were older than the other two females. The other two females were tagged as yearlings the preceding year and were thus rearing their first litter. If marking and aggression are closely associated, then higher levels of marking by older more aggressive females offers an explanation, based on age, for individual variations in marking by adult females.

As in males, marking by female mammals is not limited to the breeding season. In several canid species, Kleiman (1966) reported that females marked more frequently during oestrus, possibly indicating or signalling their reproductive condition to the male. Thiessen (1973)

believes that high frequency of scent marking observed in female gerbils, *Meriones unguiculatus*, during lactation is maternally motivated.

Marking in female Richardson ground squirrels, *S. richardsonii*, increased after parturition and peaked a few days after the young first appeared above ground (Wehrell 1973). Higher levels of marking during lactation in *S. richardsonii* and during the pregnancy-lactation period in *S. columbianus* suggest that the motivation for this behavior may have a maternal component.

A discussion of the motivational basis for scent marking in males and females is developed further in the general discussion.

Scent marking and territorial behavior. The basic unit of the social system found in Columbian ground squirrels is the group, empirically defined by Steiner (1970b) in his study of *S. columbianus* as those animals that utilize a common residence area. Within the group (composition described in the Annual Cycle section), adult males play a central role throughout the active season. Chasing intruders, primarily other adult males, from the residence area is the most noticeable activity performed by the adult males. Territoriality is suggested by this behavior.

"Territory is an area occupied more or less exclusively by animals or groups of animals by means of repulsion through overt defense or advertisement" (Wilson 1971:195). Since adult females and some yearlings are not excluded from the territory of a male Columbian ground squirrel, the male's territory might be described as a group territory (Steiner 1970b). Both Steiner's and my observations support the group territory concept that Wilson's definition of territory includes.

Betts (1973) used the term "dominance area" in place of territory; however, his description of the male's role in his population of *S. columbianus* deviated little from what I observed. Therefore, this apparent discrepancy is likely in the approach to the problem or terminology rather than in differences in behavior observed.

Changes observed in territory ownership suggested that an adult male may be attracted to and may then attempt to secure a territory where at least two or three adult females are residing. The adult females were pregnant prior to any major boundary shifts (May 5); however, it may be a good strategy for a male to obtain a territory during any season. When the probability of winning a fight is considered, a territory holder usually has a distinct advantage over an intruding male. Even if a male acquires a territory too late to inseminate any females that year, a male who is a territory holder at hibernation and then emerges on that territory in the spring appears to enhance his chances of transmitting his genes to future generations, assuming that females tend to emerge in the same areas from year to year. A male's reproductive fitness is also augmented by the protection his offspring indirectly receive from his territorial behavior.

Another reason why a male attempts to acquire and then maintains a territory in the postbreeding period may be to ensure that a suitable hibernation burrow is available. Although burrows of this type may or may not be in short supply in the habitat occupied by *S. columbianus*, Carl (1971) found increased territorial behavior in the fall in Arctic ground squirrels, *S. undulatus*, possibly to obtain a hibernation site.

Because scent marking and territoriality in adult male *S. columbianus* appear to be associated, it is possible that scent marking

may play some role in territorial behavior. Intruders were not repelled by a resident's scent mark, but some intruders appeared to become more cautious or hesitant after presumably detecting the resident's scent. In a pen study of *S. undulatus*, Watton and Keenleyside (1974) also found that a scent mark does not deter an intruder. Some repelling properties may be present in a mark because a trespasser rarely stays. Thus, a scent mark does not appear to discourage temporary invasions but may be a deterrent to permanent occupancy. If a scent mark does lower the flight threshold of an intruder then one would expect marking to be higher during the breeding period when territorial defense was vigorous than during the postbreeding period (as was observed) when territorial behavior was less intense.

The literature contains many more reports of animals using common marking points and being attracted by the scent mark of a conspecific than of any repelling properties of a scent mark. Steiner (1974) reports that an intruding male squirrel will frequently approach, sniff, and sometimes mark the same spots that the resident male regularly marks; I have observed the same behavior on a few occasions. This suggests a challenge to territory ownership.

Territory size and the proximity of neighboring groups appear to be positively correlated with marking and aggression. Males that held large territories scent marked more frequently than those males with smaller territories. It was impossible to determine if a male holding a large territory was marking more frequently because of the large area he was defending, which appeared to necessitate frequent burrow patrolling, or if increased marking was the result of more frequent aggressive encounters along more extensive boundaries. In marmots, *Marmota*

flaviventris, large territories have higher levels of conflict (Armitage 1974). Armitage also reports that territory size is influenced by the presence of nearby males.

In most instances marking was greater in colonial than in semi-isolated groups where territory size was equivalent. However, in one semi-isolated group, a male with a large territory marked two to three times more often than other males in the same situation but with territories one-half the size. Groups are spatially separated in semi-isolated situations; thus, the frequency of aggressive encounters and challenges of territorial ownership are likely reduced. Aside from influences of aggressive encounters and challenges of territory ownership, territory size *per se* seemingly affects scent marking.

The age class of males that challenge resident males for territory ownership may be the two-year-olds. Although more information would be necessary to confirm this, it seems likely that a male must attempt to acquire a territory as a two-year-old or his reproductive fitness will be reduced because of the four to five year life span of this species. I trapped several adult males that were holding small territories without females on the periphery of colonies. Perhaps these males had challenged resident males, lost and were waiting for another opportunity or another season. Some of these males were two-year-olds while others were older, possibly past their prime judging from their scarred appearance. These "peripheral" males are perhaps comparable to "satellite" males described by Barash (1973) for Olympic marmots.

LABORATORY STUDIES

HISTOLOGICAL ANALYSIS OF GLAND TISSUE

Integumentary glands from the skins of animals collected during the field portion of this study were examined macroscopically and histologically. Seasonal variation in secretory activity of the glands was emphasized, but structural features were also noted.

Methods

Suspected sites of integumentary glands were excised, dehydrated with an alcohol-benzene sequence, and embedded in paraffin (paraplast, M.P. 56° C) for two hours in a vacuum oven. The blocks were then cooled, trimmed, and sectioned on a rotary microtome at 15 micra. Tissue was mounted on slides with Mayer's albumin. Slides were stained with modified Schoor technique utilizing Harris' haematoxylin, Biebrich scarlet and Fast Green stains. Cover glasses were affixed with Permount adhesive to produce permanent slides.

A preliminary examination of the integument (see Other Body Regions section for areas examined) revealed specialized glands in oral, dorsal, and anal regions. The oral gland consisted of a gland complex that was macroscopically inseparable. However, the anal gland was composed of three glands located ventral and lateral to the anus and the dorsal glands of a field of approximately 75 individual glands. Both of the latter two gland regions were macroscopically visible.

To reduce the number of prepared slides, I mounted oral and anal gland tissue on slides in an interrupted series at every 120 micra and

dorsal gland tissue at 75 micra, because of the smaller size of the individual dorsal glands. Several complete series of each gland were also prepared to evaluate, more completely, gland structure.

Gland characteristics, which seemed related to secretory activity, were quantified with a calibrated ocular on a light microscope. Tubule diameter and epithelium height were measured on each section of the integument series that contained gland tissue. Because measuring each tubule cross section in a gland this extensive would have been extremely time-consuming, a transect method was devised. Each tubule that contacted the micrometer at 100 micra intervals through the center of the gland (dorsal to ventral) was measured for maximum diameter (outer) and epithelium height (Fig. 4). A mean value was then calculated for the entire gland.

Not all regions of one gland secrete (active budding) at an equal rate; hence, to base gland activity on the secretory activity in one section would have been inaccurate. Thus, I devised an index of secretory activity, determined by estimating the percent of the tubules where budding was occurring in each section of the interrupted series and then calculating a mean (the index value) for the entire gland.

In the oral region, sebaceous glands were subdivided, because of obvious size differences, into those near where the ducts from the underlying apocrine glands empty into hair follicles and those overlying the posterior portion of the apocrine gland. Measurements of maximum diameters were taken from all sebaceous glands in six sections, three on either side of the first duct, and from six sections at the posterior of the apocrine gland. A mean diameter of the sebaceous glands was then

calculated for the two regions. Diameters of sebaceous gland associated with the hair follicles overlying the dorsal glands were also measured. Measurements were recorded from three sections on either side of the apocrine gland duct.

When possible, the integument region containing the oral gland was removed from the squirrel's left cheek. In five animals I sectioned and analyzed both oral glands to determine if bilateral differences in gland structure and secretory activity existed; none were found.

I sectioned and analyzed several glands from various locations in the dorsal gland field to determine if the structure and secretory activity of the individual glands making up the dorsal gland field within one animal were comparable. Although variations of gland size were obvious, only minor differences in secretory activity existed; therefore, only one dorsal gland, selected at random from the dorsal gland field of each animal, was examined histologically.

Results--Gland Characteristics

Integumentary gland morphology. To provide a base for discussion of integumentary gland structure and location in terms of possible adaptive significance, I have included a brief description of the integumentary gland morphology of *S. columbianus*.

Oral gland. The oral gland occupied an area (approx. 3.2 mm anterior-posterior, 2.9 mm dorsal-ventral, 1.3 mm lateral) in the hypodermis ventral to and approximately 0.5 mm posterior to the oral angle (Fig. 5).

Epidermis of stratified squamous epithelium (approx. 20 micra

thick) and dermis consisting of dense connective tissue fibers (approx. 200-300 micra thick) composed the cutaneous gland covering. Hair follicles with associated sebaceous glands penetrated into the dermis layer; some larger hair follicles extended into the upper hypodermis. Along with the glandular complex, skeletal muscle fibers, loose connective tissue, and a few fat cells were the principal components of the hypodermis.

Lobules of the apocrine gland were encapsulated in a thin layer of connective tissue fibers, with an even thinner layer surrounding individual tubules. The gland extended into layers of skeletal muscle; each layer thickened progressively towards the posterior segments of the gland. Some of the gland lobules were separated by these layers of muscle fibers.

The apocrine gland consisted of coiled branched tubules lined by one layer of cuboidal or columnar epithelial cells. A distinct basement membrane with numerous myoepithelial cells (Fig. 6b) immediately inside surrounded each tubule.

Excretory ducts were lined with cuboidal epithelium that becomes progressively lower as the duct approached the confluent hair follicle (Fig. 6c). The diameter of the duct was markedly smaller than the secretory tubule diameter. In the immediate region of the hair follicle into which the duct emptied, the duct epithelium was stratified and was structurally similar to the follicle epithelium. Large paired sebaceous glands also emptied by way of short ducts into each hair follicle.

Three ducts located in the anterior one-half of the gland provided the pathway for the release of exudates. A layer of connective tissue,

much thicker than what enclosed each gland tubule, delineated three separate gland segments located progressively anterior to posterior and drained by separate ducts (Fig. 5).

A single cross section frequently bisected two gland regions but not all three. The presence of three distinct glands at this one location was supported by the fact that, in some cases, one gland region was actively secreting while another region appeared inactive or "spent" (Fig. 7).

Much variability (20-120 micra) in tubule diameter existed. The tubules with larger diameter (70 micra plus) were frequently located in the posterior portion of the gland and appeared to be the source of the secretory material. In some cases, the large tubules (120 micra) contained secretion but seldom appeared to be actively secreting. These large tubules might function as secretion-collecting or storage centers.

Luminal diameter depended on the condition of the surrounding epithelial cells; for instance, an exhausted gland tubule had a larger lumen (Fig. 9) because of the low epithelium than did a resting or secreting gland tubule (Fig. 10). An epithelial cell that was actively secreting had cytoplasmic protrusions on the luminal borders which indicated an apocrine mode of glandular secretion (Fig. 6a). The "blebs" that had separated from the epithelial cells appeared as granular material in the tubule lumen.

Dorsal glands. The dorsal gland field extended caudad from the scapular region approximately 90 mm in a 45 mm wide band (see Table 2) and also anterior from the scapular region in a narrow band to a small

concentration ventral to the ear (Fig. 11). Approximately 60 individual glands (Table 2) composed the dorsal gland field. A distance of 5-8 mm separated the individual glands that ranged in size from approximately 2.5 x 2.0 to 0.6 x 0.3 mm.

The pelage that covered the integument region occupied by the dorsal gland field in a live animal appeared similar to the adjacent pelage except for being somewhat oilier; however, the pelage on a prepared study skin was much oilier in the dorsal region. Removal of the cutaneous maximus muscle revealed the oval-shaped glands which appeared as light spots against the darker dermis.

Epidermis of stratified squamous epithelium and dermis of fibrous connective tissue comprised the integument overlying the dorsal glands. The integument overlying the individual gland sites was thicker (approx. 850 micra) than adjacent areas (approx. 400 micra).

Individual dorsal glands were composed of coiled tubules with short branches and a duct which opened in the free surface of the skin (Fig. 6d). Gland tubules formed a flattened mass approximately 275 micra thick that was embedded in both the lower dermis and upper hypodermis (Fig. 12a-c), approximately 500 micra below the skin surface. Cuboidal or columnar epithelium cells with basal nuclei enclosed an expanded lumen that contained granular-appearing secretions. The epithelial cells were enclosed by a basement membrane with numerous myo-epithelial cells immediately inside. A thin layer of connective tissue covered the basement membrane. As in the oral gland cytoplasmic protrusions were frequently observed on the luminal epithelium surface, indicating an apocrine-type secretory process.

With the exception of the duct, the entire dorsal gland appeared to be capable of active secretion; the branched tubules of the anterior segment appeared most active. Anterior to the origin of the duct at the posterior gland region, a segment of the gland tubules was larger (approx. 120 micra) (Fig. 6e) than the remainder of the tubules (approx. 85 micra). This segment seemed to be a collecting sinus for glandular exudate before entry into the duct. Much variability in sinus diameter of glands was noted among animals.

An abrupt transition in both epithelium height and tubule diameter marked the origin of the single secretory duct from a gland. The mean diameter of the secretory tubules was 92.9 micra in breeding males, while that of the duct was approximately 20 micra. Epithelial cells that lined the duct were low cuboidal or simple squamous. In most cases the duct extended toward the skin surface with minimal winding and contacted the free surface of the skin approximately 350 micra posterior to the gland mass. As the duct approached the skin surface, the lining epithelium changed to stratified squamous and merged with the stratified squamous epithelium of the epidermis. At the integument surface the duct diameter expanded in funnel-like fashion (Fig. 6d) to produce an aperture diameter of approximately 80 micra in a 50 micra high papilla. Ducts from the glands found ventral to the ear originated from the central region of each individual gland, whereas ducts originated from the posterior of glands found on the animal's back. A large sinus, immediately preceding the beginning of the duct, was present in glands ventral to the ear.

Anal gland. Columbian ground squirrels have three anal glands located within folds of the anal wall, two lateral and one median ventral. Under most conditions these glands were concealed within the anal orifice, but when an animal was "alarmed" the lips of the anus swelled and papillae (approx. 3 mm in diameter, 7 mm in length) were everted.

A retracted papilla was characterized by a large channel lined by heavily cornified, stratified squamous epithelium (Fig. 8). When the papilla was protruded, the channel apparently was turned inside out with the glandular elements being relocated inside the papillae. The process of protrusion and retraction of a papilla was described in detail by Sleggs (1926) in his study of the anal glands of *S. richardsonii*.

The sebaceous glands of the anal papillae resembled the sebaceous glands associated with hair follicles of the animal's integument except they were larger (approx. 700 micra in diameter). Several ducts from a alveolar sebaceous gland formed a confluence and merged with the proximal portion of the channel of the papillae. Three or four ducts, from as many elements of an apocrine gland, emptied into the channel immediately proximal to the duct system of the sebaceous gland (Fig. 8).

An expanded lumen lined by cuboidal or, more frequently, columnar epithelium that rested on a distinct basement membrane characterized the branched structure of the apocrine gland (Fig. 8). An apocrine type of secretory process was suggested by the presence of cytoplasmic protrusions on the luminal surface of the lining epithelium. The tubule masses were separated into three or four segments by layers of connective tissue. Each separate mass of tubules was drained by a duct lined by cuboidal epithelium. Ducts originated from large collecting sinuses

lined by low cuboidal, nonsecretory epithelium.

Bundles of striated muscle fibers running at various angles to one another formed a sheath that invested both apocrine and sebaceous gland elements. The inner layer of the muscle sheath extended between the layers of the connective tissue that separated the tubule masses of the apocrine gland. These muscle layers are likely involved in the extension and retraction of the papillae.

Pedal glands. Sweat glands consisting of coiled unbranched tubules were located on the volar surface of the foot pads in both sexes. A single duct from the eccrine gland emptied into funnel-shaped opening on the free surface of the pad.

Other body regions. Besides histological investigation of the oral angle, dorsal and anal regions, other integument regions were also investigated. In a publication on grooming in Columbian ground squirrels, Steiner (1973) included a figure of head regions that were frequently allogroomed and had an oily appearance. Since the attractiveness of specific head regions suggested scent implications, these regions were examined histologically. To simplify discussing and referring to these regions I utilized the same diagram and set of symbols as found in Steiner (1973) (Fig. 14).

The head regions where apocrine glands were found were ventral to arrow g in the oral angle and posterior to b ventral to the ear. In sections taken from a, b, c, d, and e only sebaceous glands associated with each hair follicle existed in the integument. Skin samples from the head regions adjacent to those listed in the preceding sentence also contained only sebaceous glands as did samples from the midventral line,

the base of the tail, the chin, and immediately posterior to the front legs. Exceptionally large sebaceous glands were associated with the large whiskers located at b. Large sebaceous glands not associated with hair follicles were found in the prepuce of the penis.

Sexual dimorphism of gland characteristics. While various oral and dorsal gland characteristics were being quantified both microscopic and macroscopic (prior to excision), features that suggested some degree of sexual dimorphism were noticed.

Oral gland. The most striking microscopic difference between males and females is the variation in diameter of gland tubules in adult females. Within one cross section, the mean range of tubule diameters was from 28.5 to 129.4 micra in females and only 41.3 to 104.5 micra in males. Tubule diameter was also significantly greater in males (male $\bar{X}$ = 70.25, female $\bar{X}$ = 53.72).

Based on measurements of dorsal-ventral, lateral, and anterior-posterior dimensions, oral gland size was found to be significantly larger (all three categories) ($P < .001$) in males than females (Table 1a). It is possible that some of the size differential could be attributed to differences in body size since adult males are generally 100-150 grams heavier than adult females.

Dorsal glands. Both microscopic dimensions (dorsal-ventral, lateral, and anterior-posterior) (Table 1b) and macroscopic dimensions (lateral and anterior-posterior) (Table 2) were significantly larger ($P < .001$) in adult males than females.

Neither the anterior-posterior and lateral measurement of the dorsal gland field nor the total number of glands in the dorsal gland field differed significantly between males and females; however, tubule diameter was significantly greater in males (male $\bar{X}$ = 88.35, female $\bar{X}$ = 56.20, $P < .001$).

The extension of the dorsal gland field ventral to the ear contains glands that are significantly larger (macroscopic) ($P < .01$) than those glands more dorsally located (Table 2). Macroscopic measurements (lateral and anterior-posterior) revealed significantly larger glands ($P < .01$) ventral to the ear in males than females (Table 2). All microscopic measurements followed the same pattern (Table 3).

Yearling gland characteristics. Since yearling animals apparently do not reproduce and were observed to scent mark on only a few occasions, only a small sample of this age class was collected. The same characteristics of the oral and dorsal glands that were quantified in the adults were also measured in nine yearlings.

Oral gland. Yearling male and female gland characteristics differed significantly in only two categories, tubule diameter ($P < .01$) and lateral dimensions ($P < .05$) (Table 4). In the remainder of the categories, including those mentioned, the mean value for the male category was larger than the corresponding female value (Table 4).

Dorsal gland. Because the dorsal glands of only one male and one female were available for histological examination, statistical analysis of the results was not performed. The results are presented in Table 4.

Discussion

Gland location and scent marking. Columbian ground squirrels have, in addition to sebaceous glands associated with each hair follicle, specialized integumentary glands located in the oral angle, dorsal, and anal body regions. Integumentary glands are found at many locations over the mammalian body (Mykytowycz 1972) and are not always limited to one gland in a single body region.

At least in some species, integumentary glands appear to be located strategically for use in scent communication. A ventral gland in the gerbil, posterolateral glands in some microtine rodents, and sideglands in some species of shrews would facilitate active or passive scent marking of the runways and tunnels through which these animals move. Sternal glands of the sugar glider and common marmoset might easily contact branches in an arboreal habitat.

Although the oral gland is not conveniently located for passive scent marking of the substrate, active marking appeared to require only minimal effort. From a walking or standing position only a slight side-tilting and a forward thrust of the head are necessary. This oral location enables an animal to scent mark after sniffing the object to be marked without a major reorientation of his body.

The dorsal gland field is seemingly not strategically located for active marking; however, the pattern of the dorsal gland field does fit the body movements used in twist marking described by Steiner (1974, Fig. 6). Active twist marking may have developed secondarily from passive nonoriented burrow marking for which the dorsal glands are conveniently located (Steiner, unpublished).

Scent marking with the anal glands in some species involves rather elaborate posturing in order to bring the glands in contact with the object being marked. The African civet, *Civettictis civetta* (Ewer and Wemmer 1974) and the African dwarf mongoose, *Helogale undulata* (Rasa 1973), actively scent mark with anal glands. An elaborate handstand posture is sometimes used by the mongoose. In Columbian ground squirrels, the anal glands may contribute to secretions deposited during anal drag marking. As described in the scent marking section (and in Steiner 1974, Fig. 6B, C), this behavior requires only a flattening of the posterior body against the substrate and a dragging of the body forward with the front legs for several inches.

Sweat glands on the volar surface of the foot pads are conveniently located for passive marking as an animal moves about. Clawing at the substrate, which frequently occurs prior to marking that same area with the oral gland, might be classified as active marking with these glands.

Sebaceous glands that are associated with each hair follicle on a squirrel's body have both scent producing and carrying potential. Large sebaceous glands located in some integument regions suggest that these regions may have greater scent producing and/or carrying capabilities than others. Since certain head regions, especially those with vibrissae, contained larger sebaceous glands and appeared more oily, sebaceous gland activity may be more intense in these regions thereby increasing their scent producing and/or scent carrying potential. Many of these head regions are extensively allogroomed (Steiner 1973). Besides sebaceous gland secretions, another explanation for the attractiveness of regions a and c (Fig. 15) is the possible involvement of secretions from the Harderian glands. In gerbils, Thiessen (pers. comm. to Steiner) found

that secretions from these glands had attractive properties for other individuals.

Gland structure and scent marking. The basic structure of the oral, dorsal, and anal glands of *S. columbianus* is similar to the oral glands of the microtine rodents (Quay 1962) and oral glands in the genus *Spermophilus* described briefly by Quay (1965), the dorsal glands of *S. lateralis* (Hatt 1926), and anal glands of *S. richardsonii* (Sleggs 1926). Therefore, only morphological features that seemed closely associated with the physical aspects of scent marking will be discussed below.

Because the three ducts from the apocrine portions of the oral gland empty into hair follicles within the anterior one-third of the gland, pressure applied to the object and the forward thrust during marking may do little to exude secretions from the gland. It seems likely that skeletal muscle layers, which envelop the posterior of the gland, and numerous myoepithelial cells may play a major role in the release of exudates.

Postures that are assumed during marking with the dorsal gland field suggest an explanation for the difference in the point at which the single duct originates from the glands located ventral to the ear (from the middle of the gland) and those glands in the dorsal integument (from posterior of the gland). Since individual glands in both locations lack the investing layer of skeletal muscle found around the oral gland, pressure against the object being marked may be necessary to exude the secretions. Glands located ventral to the ear are pressed against the substrate in a circular twisting motion that would force

secretion from the posterior duct.

The most striking feature of the anal glands is the manner in which they can be extruded and made to pulsate. Complex musculature involved in everting and retracting these glands invests both sebaceous and apocrine portions and may also force secretions from the papillae.

Sexual dimorphism of the glands. Sexual dimorphism of integumentary glands has been observed in many species of mammals (Mykytowycz 1970). In terms of gland size, males frequently have larger glands than females (e.g., gerbils, Thiessen 1973; rabbits, Mykytowycz 1965; hamsters, Kupperman 1944). If one disregards the difference in body size between the adult sexes in *S. columbianus* then males have significantly larger oral and dorsal glands. It may not be valid to do so since the body size difference in favor of males is approximately twenty percent.

It has been suggested by Mykytowycz (1970) that sexual dimorphic features, such as integumentary gland size or secretory activity, may indicate that the same glands may not be of equal importance to both sexes. When peak levels of secretory activity are compared, both oral and dorsal gland index values are at least 15-20 points higher in males than females. If frequency of marking can be used as an index of gland importance then the high levels of secretory activity associated with marking peaks supports Mykytowycz's hypothesis.

Yearling gland characteristics. The sample size of yearling oral and dorsal glands is small but the lack of significant differences between male and female oral gland characteristics suggests that if sexual dimorphism exists in adults it develops later than the yearling

stage. Interestingly, yearling gland size is very similar to that of adults. Also, relatively high values for secretory activity indicate that a potential for marking or, at least, involvement in some other type of scent communication exists.

Results--Seasonal Variations

Seasonal variations in integumentary gland characteristics

Oral gland--male. Epithelial height, secretory activity, and sebaceous gland diameter (duct region) of adult males were significantly larger in breeding than in postbreeding animals, but tubule diameter and sebaceous gland diameter in the post-duct region did not differ significantly (Table 5; Figs. 9 and 10). Epithelial height, which is a good indicator of secretory activity (Mykytowycz 1965), was positively correlated with the index of secretory activity ($r = .888$, $P < .01$). Sebaceous gland diameter (duct region) was also positively correlated ($r = .500$, $P < .01$) with secretory activity.

Secretory activity of the oral gland peaked during the breeding season (Fig. 15) when high levels of scent marking were also recorded. A significant positive correlation ($r = .576$, $P < .01$) was found between secretory activity of the oral gland and frequency of scent marking with this gland.

Oral gland--female. Although secretory activity was significantly higher during the lactation period, epithelial height did not differ significantly among the four categories of adult females (breeding, pregnant, lactating, and post-lactating) (Table 6 and Fig. 16a-d). In spite of this, a positive correlation ($r = .420$, $P < .01$) existed

between secretory activity and epithelial height. Although sebaceous gland diameter (duct region) was significantly larger in the three postbreeding categories, post-duct sebaceous glands did not differ significantly throughout the entire season (Table 6). A positive correlation was found between secretory activity and diameter of sebaceous glands in the duct region ($r = .345$, $P < .02$).

Secretory activity peaked during the pregnancy-lactation period (Fig. 17) when scent marking was observed most frequently.

Dorsal gland--male. Dorsal gland characteristics of adult males exhibited the same pattern of seasonal variations as found in the oral gland. Epithelial height, secretory activity, and sebaceous gland diameter were significantly greater in breeding than post-breeding animals, but tubule diameter did not differ significantly (Table 7 and Fig. 12a-c). Both epithelial height and sebaceous gland diameter were found to be correlated with secretory activity ($r = .531$, $P < .01$; $r = .596$, $P < .01$ respectively). Secretory activity of the dorsal glands peaked during the breeding season (Fig. 18) when most instances of marking with the dorsal gland field were noted.

Dorsal gland--female. As in the oral gland of females, no significant change in epithelial height was recorded; although, significantly higher secretory activity existed during the pregnancy-lactation period (Table 8, and Fig. 13a-d). Sebaceous gland diameter was significantly greater during the pregnancy period, while tubule diameter was somewhat larger in postbreeding animals (Table 4). Secretory activity was positively correlated with epithelial height ($r = .640$, $P < .01$), but not with sebaceous gland diameter. As with the

oral gland, secretory activity of the dorsal gland was maximum during the pregnancy-lactation period (Fig. 19).

Anal gland--male. Tubule diameter did not differ significantly between breeding and postbreeding animals (Table 9). Epithelial height and secretory activity were both greater during the breeding season; however, only epithelial height was significantly greater (Table 9). High variability in secretory activity values from the breeding season sample may have contributed to an insignificant "t" value.

Anal gland--female. Comparisons of breeding with postbreeding females (pregnant, lactating, and post-lactating) revealed significant differences in epithelial height and secretory activity, but not tubule diameter (Table 10). The postbreeding category contained the significantly larger means.

Seasonal variation in testis weights. Maximum testes weight of adult males was found in the animals that had just emerged from hibernation (Fig. 20). Subsequent samples revealed a steady decline in testes weights with a low being reached in early June and some recrudescence occurring in late July. Active spermatogenesis was present in seminiferous tubules of the males collected early in the active season, but no spermatogenesis or interstitial cell proliferation was apparent in midsummer samples or in the late summer period of augmented testes weights (Fig. 21a, b, c).

Discussion

Seasonal variations in integumentary gland characteristics. As with scent marking, cyclic variations in gland activity have been reported. Increased gland activity during the breeding season was found for water voles, *Arvicola terrestris* (Stoddart 1972), for woodrats, *Neotoma fuscipes* (Linsdale and Tevis 1951), for male rabbits, *Oryctolagus cuniculus* (Mykytowycz 1965, 1966b), and for male black-tailed deer, *Odocoileus hemionus* (Muller-Schwarze 1972). The latter two researchers found simultaneous peaks in gland activity and scent marking. Data collected in this study parallel these findings.

Even though gland activity peaked in male *S. columbianus* during the breeding season, secretory activity did not fall to a zero level in the post-breeding period. Scent marking was still performed, although not as frequently during this time; thus, one would not expect a complete cessation of gland activity.

Reports of seasonal patterns of gland activity in females are few. Some investigators have suggested that gland activity may peak during oestrus when higher levels of marking were recorded. Peaks in gland activity have been found outside the oestrus period in two species of shrews, *Blarina brevicauda* (Pearson 1946) and *Cryptotis parva* (Kivett 1971). The possibility of a maternal component of motivation for the higher levels of scent marking, as presented in the Scent Marking section, is supported by the peaks in gland activity that also occur in the pregnancy-lactation period in Columbian ground squirrels.

Sebaceous gland exudates may provide a transfer medium for secretion from underlying apocrine glands (Dryden and Conaway 1967).

Sebaceous glands may function similarly in Columbian ground squirrels since large sebaceous glands were found adjacent to duct openings of the apocrine glands (oral and dorsal). The positive correlation between secretory activity of the apocrine glands and diameter of sebaceous glands in the duct region indicates that differential stimulation of these sebaceous glands and sebaceous glands in other areas might be occurring, since diameters of sebaceous glands in adjacent integument regions remain relatively constant throughout the season. Differential stimulation of sebaceous glands has been shown for the supracaudal gland of the guinea-pig (Martan and Price 1967) and the costovertebral gland of the hamster (Hamilton and Montagna 1950).

Peaks in anal gland activity during the breeding season as Mykytowycz (1966a) found for rabbits, *Oryctolagus cuniculus*, and for hares, *Lepus europaeus*, do not appear to exist in the anal gland complex of *S. columbianus*. Trends were found that larger sample size might substantiate, but because of the difficulties in sectioning this tissue and determining if this gland was involved in active marking, emphasis was placed on the oral and dorsal glands.

Seasonal variations in testis weight. Although maximum testis weight was found in the sample of males collected within a few days after their emergence from hibernation, approximately three days before the adult females, breeding was not completed until approximately two weeks later. After emergence from hibernation testis weight dropped rapidly. In *S. undulatus*, Mitchell (1959) found that sperm was still present in the epididymis even though the testes had begun to atrophy. A similar pattern, which would account for the continued breeding

capabilities of the males despite atrophy of the testes, may exist in *S. columbianus*.

The late summer recrudescence of the testes is apparently in preparation for breeding, shortly after emergence from hibernation. Shaw (1926b) found that in *S. columbianus* the testes increased in size during hibernation and at emergence were maximum in size and scrotal. Similar enlargement of the testes prior to onset of hibernation have been reported for *S. tridecemlineatus* (Wells 1935), *S. beecheyi* (Tomich 1962), *S. beldingi* and *S. lateralis* (McKeever 1965). Since the timing of reproduction is important, especially in the short season that many Columbian ground squirrels experience, late season testis development may be essential.

EFFECTS OF EXOGENOUS TESTOSTERONE ON GLAND CHARACTERISTICS OF ADULT MALES

In the sample of males collected in 1972 secretory activity of the integumentary glands (oral and dorsal) peaked during the breeding season. If one can assume that testosterone levels are high in a reproductively active male then it seemed possible that testosterone might be influencing gland activity. An experiment was designed to test this supposition.

Methods

Fifteen adult male *S. columbianus* in equivalent reproductive condition (one week postbreeding) were live-trapped May 29-31, 1973, and returned to Edmonton. On June 1, 1973, ten of the fifteen males were bilaterally castrated and allowed to recover for three days in individual

cages. Five of the castrates were then randomly selected and given subcutaneous injections of testosterone propionate (TP) suspended in peanut oil (2 mg/injection) daily. Only the oil vehicle was administered to the remaining five castrates. The intact males became a control group (no injections). The control group served as a base line of gland activity for comparison with the castrates and TP-treated groups. After fourteen days of hormone treatment I sacrificed all fifteen animals and fixed the entire skin in A.F.A. for histological analysis.

Results

Oral gland. Epithelial height, secretory activity, and sebaceous gland diameter (duct region) were found to be significantly greater in the testosterone-propionate treated group (Table 11). In only one parameter, tubule diameter, did the castrates (without TP) differ significantly from the control group.

Dorsal gland. A significant increase in secretory activity and sebaceous gland diameter were found following testosterone-propionate treatment (Table 12). Although the castrate group (without TP) exhibited a significantly smaller tubule diameter than the castrates (with TP) or the control group, epithelial height did not differ significantly between the testosterone treated and control group.

Discussion

The hormonal control of secretory activity of integumentary glands has been investigated in several mammalian species. Atrophy of sebaceous glands after castration has been reported in the male gerbil

(Thiessen *et al.* 1968), and guinea-pig (Martan 1962), and the shrew (Dryden and Conaway 1967), while testosterone stimulated the sebaceous glands in the same species. Based on extensive information there is general agreement that androgens increase the size and functional capacity of sebaceous glands (Strauss and Ebling 1970). The effect of testosterone on apocrine glands has not been as extensively investigated. However, Mykytowycz (1962, 1965) and Wales and Ebling (as cited in Strauss and Ebling 1970) found that castration inhibited and testosterone stimulated apocrine gland development and activity.

Somewhat similar patterns as mentioned above were found in male Columbian ground squirrels. Although secretory activity of both apocrine and sebaceous glands increased after administration of testosterone-propionate to castrate animals, gland characteristics of the castrate group (without TP) and the control group remained similar. Prior studies (mentioned above) indicated that castration might result in atrophy of the skin glands. An explanation for this discrepancy is that testosterone levels in the postbreeding animals used in the experiment were probably extremely low; thus, testosterone levels and related gland activity were not greatly reduced by castration. Gland characteristics of the castrate and control group approximated the gland characteristics of the postbreeding male category (see Seasonal Variation in Integumentary Gland Characteristics section). This supports the probability of low testosterone levels existing in males used for experimentation. Gland characteristics of the testosterone treated group and breeding male category were also similar.

Since integumentary glands seem to be stimulated by testosterone, the seasonal pattern of testes weight, with maximum values being recorded

from breeding males (see Seasonal Variations in Testes Weight section), suggest that augmented testosterone production may occur at this time. It also seems plausible that scent marking by males may be influenced by the same hormone. The positive correlation found between marking frequency and testes weight in this study has also been reported by Thiessen (1968) for gerbils.

CONTROLLED PEN EXPERIMENTS ON SCENT MARKING AND RELATED BEHAVIOR

General Methods

Behavior experiments during the summers of 1973 and 1974 were conducted in a rectangular outdoor pen (4.9 x 12.1 m) that was separated into two equal sections by a divider which contained a removable access passage (Fig. 22). The concrete floor of the pen was covered with dirt to a depth of 15 to 20 cm around a central mound approximately 45 cm high and then sodded; this simulated a natural substrate in which the squirrels could burrow. Two nest boxes plus food (Vitamite pellets) and water were provided *ad libitum* in each section. Observations were made with binoculars from a concealed vantage point above and to one side of the pens.

Prior to the experiments conducted in 1973 behavior observations were made for two weeks on animals of known social status (recorded in the field) to determine if the pen situation was markedly affecting behavior patterns. Since dominance relationships remained constant, scent marking was observed, and one female successfully reared a litter of four, I concluded that the pen was not adversely influencing behavior

that would be important in future experiments.

During experimentation marking pegs were positioned in the pen prior to morning emergence of the animals. Because the central mound was the focal point of the animal's activity, the pegs were arranged to center on the mound (Fig. 23). Marking and other behaviors were then recorded from the time the first male emerged until two hours had elapsed. I used this observation period because field observations indicated that maximal activity, including scent marking, occurred during the first two hours following morning emergence. As in the field the pegs were collected immediately after the observation period and cleaned.

Scent Marking and Social Status

Higher levels of scent marking by adult males that were dominant within their group indicated that frequency of scent marking might be related to social status. To investigate this hypothesis I designed the following experiment.

Methods. Four groups of two males and two females were selected. Two groups had previous social experience together and two were selected at random from animals with no known social contact with one another. These animals had been captured in 1972 and had been in hibernation in the Laboratory Animal Service facilities at the University of Alberta. The squirrels were aroused in April of 1973; thus, their normal cycle of reproductive activity was completed before this experiment was initiated in June of 1973.

After a group was introduced into one section of the roof pen, observations (three observation periods) to determine social status of

the males were made. Dominance was based on the same criteria as described in the Field Methods section. Females were included to simulate more closely group composition as it exists in field conditions. The subordinate male was then given subcutaneous injections (2.0 mg) of testosterone propionate (TP) three times each week for a two-week period with the intention of producing a reversal in male dominance. Scent marking and other relevant data were recorded daily in the time period mentioned in the General Methods section.

Results. The first detectable changes in the behavior of the subordinate male were noticed on the second or third day after the inception of hormone injections. Slight increases in scent marking by the subordinate male and marked increases in male-male encounters that were initiated by the subordinate were the general pattern. Prior to this time almost all interactions had been initiated by the dominant male. Fighting between the males increased until a reversal in dominance occurred, usually by the fifth day ($\bar{X}$ = 5.8 days).

After the dominance reversal scent marking of both the ground and pegs by the "new" dominant increased while marking by the "new" subordinate decreased, both significantly ($F = 6.4$, $P < .05$; $F = 33.9$, $P < .001$, respectively) (Table 13). Marking by the "new" dominant decreased only slightly over the next five days. Marking data used in the statistical analysis encompassed a 10-day interval, five days prior to and five days after the dominance reversal.

Discussion. Under field conditions dominance among males that hold territories is primarily site-dependent in that a male who is within his own territory will usually be dominant to neighboring males. The

(4.9 x 12.1 m) pen was apparently too small to allow two territories to be established; therefore, one male could not escape and a dominant-subordinate relationship developed.

The higher levels of marking recorded for the dominant animals in this study have been reported in several other mammalian species (e.g., rabbits, Mykytowycz 1968; marmosets, Epple 1970; African dwarf mongoose, Rasa 1973). Increased marking by a male after becoming dominant and decreased marking by a male after becoming subordinant has been found in gerbils (Thiessen 1973). Thiessen stated that these marking changes were independent of previous marking levels; this also appears to be true in male *S. columbianus*. One cannot entirely separate the possible influences of TP and social status reversals on marking frequency: however, marking by the subordinate began increasing three days after TP was administered and continued to increase gradually until the sharp increase as status was reversed. Also, the former dominant's marking decreased after he relinquished the dominant position even though hormone levels may not have changed. This indicates that both social status and testosterone influence marking.

Increased marking after androgens were administered has been reported in gerbils (Thiessen *et al.* 1968), but, in this experiment increased marking cannot be attributed to androgens alone because increases in aggression and marking occurred simultaneously and increased marking may have resulted from increased aggression. However, androgens may be responsible for increased aggression exhibited by subordinate males and subsequent dominance reversals. Guhl (1961 as cited in Whitsett 1975) presented a number of studies showing that aggression increased when testosterone was administered. In a recent study Vandenbergh (1971)

obtained similar results in hamsters (*Mesocricetus auratus*).

If one considers the dominant male in the pen situation as the territory holder and the subordinate a male who does not hold a territory, then increased scent marking by the adult males after acquiring dominant status is in agreement with field observations of territorial behavior. A decline in marking by a male that has lost a dominant position is comparable to a male in the field that has lost a portion or all of his territory (see Scent Marking and Territorial Behavior section).

Scent Marking and Social Setting

Since the emergence from hibernation of adult females coincided with a sharp increase in male-male aggression and scent marking, I attempted to determine the female's role in these major behavioral changes.

Methods. I tried to simulate the emergence from hibernation sequence of the adults, based on field observations, by introducing the squirrels into the pens in a similar sequence. By doing this I hoped to determine the influence of an adult female on the behavior of adult males in a situation of male rivalry (see Combination A below). To evaluate the possible effect of a female in the absence of male rivalry, I used Combination B. Although the first six days of Combination A are identical to Combination C, it would have been necessary to subdivide A for statistical comparison to B; thus C was included in the design.

Eight sets of three animals (two adult males and one adult female) with four sets having previous social experience together were selected. In the remaining four sets none of the animals had any known, previous

social contact with other members of a set. Both the set of animals and the order of use of combinations A, B, and C (listed below) were randomly chosen; each set was involved in all three combinations.

	Days 1 to 3	4 to 6	7 to 9
A.	Resident ♂ added	Nonresident ♂ added	♀ added
B.	Resident ♂ added	♀ added	
C.	Resident ♂ added	Nonresident ♂ added	

In each combination the resident male was introduced into the pen on the afternoon prior to the observation period on the next morning (Day 1), but both the nonresident male and female were introduced ten to twenty minutes before the animal(s) in the pen emerged in the morning (Day 4 or Day 7). After introduction each animal remained in the pen until the entire sequence was completed. Data were recorded for three days for each single animal or combination of animals before the next animal in the sequence was introduced (e.g., Combination A required nine consecutive days; three days of observation with the resident male alone, three days with the resident plus the nonresident male, and three more days with the resident male, the rival, and the female).

Prior to the experiment, dominance status of the males was determined by simultaneous introduction of one pair into a neutral arena (1.5 x 6.0 m). The more aggressive or the winner of the fights during the next hour was considered the dominant. The dominant animal of the pair was used as the resident (first introduced) and the subordinate as the nonresident male for each combination in the experiment. This was

done to "reinforce" dominance of the resident, thereby reducing the possibility of dominance reversals after experimentation began because both males of a pair may have been potential territory holders in field conditions. Since I was primarily interested in behavioral changes of a territorial dominant male, it was desirable to prevent, if possible, dominance reversals.

In order to maintain a uniform reproductive condition of the animals throughout this four-month experiment, I administered subcutaneous injections of TP to the males and estradiol benzoate (EB) to the females. Each animal received hormone injections (2.0 mg of TP or EB/day) for five days prior to and every second day after introduction into the pen.

To make possible the running of two simultaneous combinations, I placed a visual barrier between the halves of the pen. Frequency of scent marking (both pegs and ground), aggression, clawing of ground and other relevant behavior were recorded on prepared data sheets by two observers. Each observer selected at random four of the eight sets and recorded information for all combinations of those four sets.

Results. Although mean values for the three-day interval in each combination were statistically compared, in some instances I felt that means for one day, especially on the day another animal was introduced (e.g., Days 4 and 7) better exemplified short-term behavioral changes. Mean values for three-day intervals give a better idea of total effects or duration of effects, but they appeared to be, in some cases, having a diluting effect on maximum responses. Therefore, I have included day-to-day changes when they seemed pertinent. All daily means are presented

in the Appendix (I, II, III).

During the first three-day interval (all combinations) when only the resident male was in the pen, the highest level of scent marking was recorded on Day 1 with somewhat of a decline on Days 2 and 3 (Fig. 24a, b, c).

In Combination A, a significant increase in scent marking by resident males was recorded after the introduction of a nonresident male (first 3-day interval compared to second) (Table 14). The highest level of male-male encounters (chases and fights) and clawing, as well as significant increases in scent marking (Day 3 $\bar{x}$ = 17.4, Day 4 $\bar{x}$ = 31.6) were recorded on the first day the nonresident male was added. Frequency of scent marking and clawing by resident males paralleled decreases in aggression over the next two days (Days 5 and 6).

After introduction of the female (Day 7) in Combination A, significant increases in male-male encounters (Day 6 $\bar{x}$ = 4.8, Day 7 $\bar{x}$ = 16.5) and clawing (Day 6 $\bar{x}$ = 1.7, Day 7 $\bar{x}$ = 5.4) by the resident male were recorded; however, scent marking by resident males increased (Day 6 $\bar{x}$ = 26.2, Day 7 $\bar{x}$ = 32.0), but not significantly. Besides more frequent aggressive encounters, the duration of each chase was longer and often ended in a fight. Fighting between males was infrequent on the Day 6.

Scent marking and clawing by resident males increased significantly (first three-day interval compared to second) after the introduction of a female (Combination B) (Table 14). In six of the eight sets the resident male mounted the female and attempted to or did copulate with the female in both Combinations A and B.

A significant negative correlation ($r_s = -.885$, $P < .01$) (Spearman Rank Correlation) was found between frequency of scent marking by nonresident males and frequency of male-male encounters in Combination A. As soon as the resident male established his dominance and became somewhat more tolerant of the nonresident male's presence, marking by nonresident males increased. If the nonresident emerged before the resident or if the resident entered one of the houses while courting the female, marking by the nonresident male was higher than when the resident was visible.

Frequency of scent marking (Day 3 $\bar{x}$ = 17.4, Day 4 $\bar{x}$ = 25.5) and clawing (Day 3 $\bar{x}$ = 1.6, Day 4 $\bar{x}$ = 5.4) (Treatment C) increased significantly after the nonresident male was introduced when Day 3 was compared to Day 4, but not when the means for the three-day intervals were compared (Table 14). Although the treatment sequence was selected at random, Combination A was run prior to Combination C in six of eight cases. Previous experience may have influenced the apparent increased tolerance of the males to one another in Combination C.

When scent marking by the resident male was examined on a day-to-day basis, a similar pattern of increased marking on the day another animal was introduced was found in each combination (A, B, and C) (Fig. 24a, b, c).

Discussion. Many species mark more frequently after encountering members of their own sex than after encounters with members of the opposite sex (Ralls 1971). However, results of this controlled experiment indicate that the presence of a reproductively active female may be influential in initiating and maintaining high levels of male scent

marking and male-male aggression.

Based on field observations, I expected a greater increase in marking by the resident male after a female was introduced in a situation of male rivalry (Combination A) than was recorded. The limited increase in marking may be due to an upper limit of scent marking having already been reached before the female was added. It is likely that the small enclosure forced the males to interact more frequently than would have occurred in the field. Under field conditions the defeated male (non-resident) would have probably escaped. If scent marking and aggression are associated, and they appear to be to some extent in this species, then the levels of marking recorded before the female was introduced may have been near the resident's upper limit of marking because of frequent aggressive male-male encounters. In reviews of scent marking Ralls (1971) and Johnson (1973) concluded that an association between marking and aggression exists in a large number of species.

Perhaps the significant increase in male-male encounters (Combination A) is a better indicator than scent marking of the female's influence on a resident male. If the female's presence was neutral, then one would not expect increased male-male aggression.

The fact that marking by the resident male was sustained at a high level throughout the three-day interval (Days 4-6, Combination B) while dropping off after Day 4 (Combination C) suggests that males and females may affect marking responses differently. If marking is affected equally by the presence of any squirrel then one would expect comparable levels of marking responses to both males and females.

A higher level of marking on the first day a resident male was in the pen might be attributed to lingering odors from previous experiments

or to the novelty of the situation. Increased marking after being introduced into a new cage has been reported in the tree shrew, *Tupaia belangeria* (Martin 1968) and the bushytailed woodrat, *Neotoma cinerea* (Egoscue 1962), with maximum marking occurring at some moderate level of novelty (Johnson 1973). In the field, marking seems to be higher during the "territory acquiring" period than in an established territory where a male has likely established his own scent (see Scent Marking and Territorial Behavior section). This could also account for the resident's higher level of marking on the first day in the pen.

Since clawing the ground frequently preceded marking, this behavior may be associated, in some manner, with scent marking. Modification of the substrate by clawing may provide a more suitable site for marking or, as Eisenberg and Kleiman (1972) suggest, the scratched-up earth may attract an animal's attention through the smell of fresh earth or by making the area conspicuous. The resident male did claw more frequently in the presence of another animal. The scratched earth may have attraction value or the squirrels may have been actively marking with secretions produced by the pedal glands. Clawing before marking has been previously reported in Uinta ground squirrels, *S. armatus* (Balph and Stokes 1963), and in Columbian ground squirrels (Steiner 1970, 1974).

The suggestion that animals scent mark when dominant to and/or intolerant of another animal (Ralls 1971) seems applicable to the resident-nonresident male situation in this experiment. Even the slight increase in marking by the nonresident as the resident became less aggressive demonstrates how closely scent marking seems to be related to social status and aggression levels. Of course, familiarization may have

influenced these behavioral changes.

The possibility was considered that any Columbian ground squirrel, not just a reproductively active male or female, might influence marking by the resident male. I decided that the only animals that approached neutrality and could be used in such an experiment were juveniles or yearlings, particularly females since they are not a potential challenge to a male. Because field observations indicated that both of these age classes play a neutral role throughout most of the year, especially the breeding season, I concluded that their influence would be slight. Also, yearlings emerged from hibernation three to four days after the adult females, at which time no further increase in scent marking by adult males was recorded. However, later in the season, the yearling males are chased by resident males (see Description of Annual Cycle). Because of these factors and time limitations for running the experiment, a "neutral" animal was deleted from the experimental design.

Differential Responses to Scent Stimuli

If scent marks convey some type of information to conspecifics, one might expect an animal to be able to recognize another animal's scent mark as being different from his own. If so, an adult male may respond differently to his own scent mark than to the scent mark of a strange male. Whether the response is in the form of an immediate change in behavior likely depends on the message detected in the scent mark.

Methods. The responses of one adult male to his own and a stranger's scent were recorded in a room (7 x 9 m) where he had been housed singly in a shoe box cage and allowed daily freedom (approx. 2 hr/day) for six months prior to this experiment. This animal had been

captured as a juvenile and tamed by extensive handling to a point where the presence and activity of the experimenter did not adversely affect his behavior.

Three marking pegs were taped to the floor near the center of the room in a line with 25 cm between each peg; the male was released at a standard location, an equal distance from each peg. One peg (referred to as "stranger") had been scent marked (oral gland) that same morning by another adult male (not always the same male) which the experimental male had never contacted; another, referred to as "own" had been scent marked the previous day by the experimental animal, and a third was unmarked (neutral). The position of the pegs was shifted in a regular sequence daily. For example, if the sequence from left to right was "own"-"stranger"-"neutral", on the next day it would be "neutral"-"own"-"stranger".

Beginning with the release of the subject, the total number of times each peg was marked during four consecutive five-minute intervals was recorded. The twenty-minute interval was subdivided to expose any temporal differences that might exist in the male's response to the scent. Each test was run between 10:00 and 11:00 AM for ten successive days during two periods: (1) while the male was reproductively active; (2) one month after the first test period when the male was no longer reproductively active (postbreeding).

Results. The subject had been allowed to scent mark a peg that was covered with his own scent mark for several days before the "strange" scent was introduced. During the period prior to presentation of the strange scent mark, marking by the subject was not quantified, but his

marking appeared to be less frequent and almost entirely limited to peg marking.

When the "strange" scent was presented for the first time, the male sniffed the "strange" peg, immediately flared his tail, and then vigorously marked the peg (strange) several times. Tail flaring was never observed when his own scent was investigated. In the field, squirrels flared their tails when excited and in aggressive situations (Steiner 1970b; personal observations). Next, what can best be described as a marking frenzy occurred; everything in the room that projected enough to allow contact with the oral angle was marked. Including the three pegs and objects in the room, the male marked a total of sixty times during the first five-minute interval. Although peg marking remained consistently high, scent marking of other objects in the room declined somewhat during subsequent days.

Besides the total number of times that pegs were marked during the breeding season differing significantly from postbreeding season totals, several differences in the response to the three pegs were also found (Table 15). During the breeding season of the subject the peg that was covered with the strange scent was marked significantly more frequently in each five-minute interval (combining the third and fourth) and in total (Table 15). The third and fourth intervals were combined to facilitate analysis because Sokal and Rohlf (1969) caution against using expected frequencies that are less than five. Only the third and fourth intervals combined and the totals differed significantly in the postbreeding tests (Table 15). When the postbreeding totals were compared, the "stranger" value differed significantly from the "neutral" value but

not from the "own" value. When marking in equivalent five-minute intervals (breeding vs. postbreeding) was compared all except "own" and "stranger" during the first five-minute interval were significantly greater during the breeding season (Table 16). The total number of times that pegs were marked during the breeding season (418) differed significantly ($X^2 = 57.33$, $P < .005$) from the postbreeding total (225).

Discussion. The ability of mammals to discriminate by olfaction has been demonstrated at several levels. This ability ranges from discrimination of sex or reproductive state in many species to discrimination of individuals in a few instances (e.g., mice, Bowers and Alexander 1967; gerbils, Dagg and Windsor 1971). Male *S. columbianus* appear to be able to discriminate between their own scent mark and the scent mark of a strange male. The somewhat aggressive response of the male after investigating the strange scent (increased marking and excitement) suggests that a squirrel may acquire more information from a scent mark than just recognizing the scent as being different. Since a territorial male in the wild responds in a similar manner after detecting the scent mark of a strange male within his territory, threat and/or adult maleness, either of which might elicit an aggressive response, may be conveyed in a scent mark. The level of discrimination need not be one of individuality to explain the responses to the scent that were observed.

Of course, a male may respond in the same manner to any scent mark that is not his own. Because of the difficulty in obtaining pegs marked by adult females, I was not able to investigate the response of an adult male to a female's scent mark. But if one assumes that the same scent signal that is conveyed during greeting, which also involves the

oral gland, is contained in a scent mark then I would not expect an aggressive response by a male to a female's scent mark. Adult males seldom respond aggressively after greeting a female; on the other hand, aggression is common after two males greet. When greeting a female, a male may recognize a "femaleness" factor or the absence of a threat component.

Higher levels of marking during the first test period when the test male was reproductively active coincided with marking data collected in the field. The greatest amount of marking was recorded in the first (5 min) interval in both the breeding and postbreeding test periods, but marking during subsequent (5 min) intervals declined more rapidly in the postbreeding period. If animals mark more frequently when they are intolerant of another animal as suggested by Ralls (1971), then the more sudden decline in marking in the postbreeding period could indicate that increased tolerance may exist during this period. I observed a lower level of male-male aggression and increased tolerance of trespassing males by resident males in the field after the breeding season (see Description of Annual Cycle).

GENERAL DISCUSSION

MOTIVATION AND CONTROL OF SCENT MARKING

Many inferences have been made pertaining to why animals scent mark. Ewer (1968) stated that the factors which evoke marking are unclear; however, Ralls (1971) concluded that particularly high levels of marking are primarily associated with aggressive tendencies. Eisenberg and Kleiman (1972) concurred with Ralls and attempted to account for low levels of marking with an odor field hypothesis. They proposed that an optimum odor field, composed of a combination of scent stimuli from the individual, the environment, and conspecifics, provides an optimum level of security for an animal. Any modification of this odor field elicits an appropriate behavioral adjustment. Besides aggressive behavior, Johnson (1973) classifies sexual behavior and novel stimuli as factors that influence marking.

Although the level of analysis of marking may be obvious in many instances, Eisenberg and Kleiman (1972) stated that too often people neglect to define the level when discussing the motivation of marking. These authors felt that on one level an analysis can be made of those external or internal stimuli which influence the frequency of marking (e.g., manipulation of the environment or hormonal control). At another level Ralls (1971) suggests that the motivation of marking can be investigated by associating marking with a stimulus or behavior of a known motivational state. In this section I attempt to determine the motivation for marking in *S. columbianus* by utilizing different levels of analysis.

If one considers the motivation of scent marking in Columbian ground squirrels by using temporal associations, then scent marking in both males and females appears to be motivated, at least in part, by agonistic tendencies. When territorial behavior is vigorous and levels of aggression are high, the level of scent marking is also high. In adult males, the onset of territorial behavior, high levels of scent marking, and aggression coincided with the emergence from hibernation of adult females. It seemed as if the presence of the females actually triggered the noticeable behavior changes in males. The onset of territorial behavior in adult females was more gradual, apparently not evoked by a single external stimulus as may be the case for males.

When internal stimuli are considered in adult males, hormones seem to influence, either directly or indirectly, the frequency of scent marking, because marking and aggression increased after administration of testosterone. This indicates that testosterone may be involved in early spring behavior of males. However, one might not expect such sudden behavior changes (as mentioned above) on the day that the females emerge from hibernation unless the males were already physiologically prepared. If the females' presence results in the release of androgens, then a more gradual increase in marking and aggression might be expected. Even though androgen levels may not be directly reflected in testis weight, maximum values of testes weight were found in males immediately after spring emergence, indicating that a high potential for androgen production may exist at this time. McMillin *et al.* (1974) for white-tailed deer (*Odocoileus virginianus*), Neaves (1973) for the rock hyrax (*Procavia habessinica*), and Hurlbut (1971) for cottontail rabbits

(*Sylvilagus floridanus*) report that increased serum testosterone coincided with increased testicular size. Testosterone may produce a capacity for high levels of aggression and marking; the emergence of the females from hibernation may activate these behaviors.

The onset of territorial behavior and marking in females early in pregnancy and the continuation of this behavior throughout lactation may be influenced by hormones associated with pregnancy and lactation. Johnson (1973) cited a few instances of females marking during oestrus but stated that data on marking by females are rare. Information pertaining to the influence of estrogen and progesterone on integumentary glands is also limited; however, both estrogen and progesterone have been reported to stimulate scent gland activity in some mammals (see Strauss and Ebling 1970). In a series of studies where both marking and hormonal influences were investigated, marking in female gerbils peaked during lactation (Thiessen 1973), but marking behavior and secretory activity of the ventral gland were apparently not under control of estrogen and progesterone; exogenous testosterone elevated marking and increased gland size (Thiessen and Lindzey 1970). Thiessen also suggests that marking and gland activity in the female gerbil may depend on androgens from extragonadal sources. This may also be the case for female *S. columbianus*.

Marking by adult males persists beyond the breeding season, although at a reduced level (see Scent Marking section). Again, marking during this period may be motivated by aggressive tendencies, but reduced aggression, coinciding with less vigorous territorial behavior, may be associated with lower levels of marking. If testosterone production is lowered when testes weight is reduced in the postbreeding period, then

reduced levels of marking may reflect, to some extent, the levels of this hormone. As in the breeding season, marking and aggression seem inseparable.

A similar explanation for the motivation of marking in post-breeding males may be applicable to females when they are not territorial during the breeding and post-lactation periods. As in males, reduced levels of aggression, likely associated with the absence of territorial behavior, may be reflected in infrequent marking.

POSSIBLE FUNCTIONS AND MEANINGS OF SCENT MARKS

Schultze-Westrum (1965) listed fifteen possible functions of scent produced by mammals. This list will likely grow as scent communication is investigated further. In this section the possible functions and meaning of scent marks from secretions produced by the oral, dorsal, and anal glands are considered.

Oral Gland Scent Mark

Since scent marking by adult males occurred more frequently during the breeding season when territorial behavior was most intense, marking may serve some purpose in this behavior. A male defends his territory, primarily, against intruding males; thus, if a message(s) is conveyed by a scent mark, it may be directed to other adult males. When a territorial male detects a strange scent mark in his territory he sometimes claws vigorously and then marks the spot where the strange scent was located several times. This hints that an intruder's scent may have a "threat" or "adult maleness" meaning.

Threat has been suggested as a function of scent in a variety of

mammals (e.g., sugar glider, Schultze-Westrum 1965; mongoose, Rasa 1973; black-tailed deer, Muller-Schwarze 1971; beaver, Aleksiuk 1968). In the laboratory, scent that accumulated on one's hands while handling *S. columbianus* produced threat displays, threat postures, and attack in eight *S. franklinii* that were born in captivity. These same squirrels could be readily handled by the experimenter if the Columbian ground squirrel scent was removed by thorough washing.

As was pointed out in the Scent Marking and Territorial Behavior section, a scent mark does not deter an intruder. If detecting a resident's scent mark only results in an intruder becoming more hesitant, cautious, and more likely to flee as a threat or adult maleness meaning might achieve, it still serves a useful purpose in territorial behavior.

Another possible message conveyed by a scent mark is that of individual identity. A male could then differentiate among the scent marks of neighboring males; marks of non-neighboring males would be recognized as being those of unfamiliar animals. Even when individuality alone is conveyed, if an animal encounters a scent mark often enough and learns to associate a certain scent with an attack and an ensuing fight that he frequently loses, then a scent mark could take on aversive properties. A situation such as this would be established between neighboring males. Peripheral (non-territory holders) males that are attempting to secure a territory or a non-neighboring male might not associate the resident male's scent with any unpleasant previous experience if they have not contacted this male before. Because these males might trespass further and be less inclined to flee, confrontations with resident males may occur more frequently. Although no quantitative information is available, a resident male would seemingly fight more

readily with strange than with neighboring males unless, of course, a neighbor was attempting to encroach on a portion of his territory.

Whatever the content of a scent message, temporal persistence of a mark, considered by Mykytowycz (1972) to be its most important feature, would allow the message to be transmitted in the absence of the resident male. This could reduce the number of overt fights and possible injuries because an intruder might be aware that he is trespassing on an area occupied by an adult male and may be more inclined to flee.

If a male's physical presence, which would frequently result in fighting, was the only method of informing an intruder that a territory was occupied, then energy demands would be greater than if some other method of communication were utilized. Betts (1973) states that territoriality in *S. columbianus* is uneconomical because of the high energy demands of territorial behavior. This does not seem to be the case in the population I studied, but it does seem plausible that energy expenditure might increase if scent marking was not utilized.

Although I did not observe the response of an adult female to the mark of another female, higher levels of scent marking when the females are territorial suggests that their scent mark may also convey a threat message.

Dorsal Gland Scent Mark

Many mammalian species have scent glands at more than one body location and sometimes mark with these glands singly or in combination. When Columbian ground squirrels twist mark, both the oral gland and the dorsal gland field, in many instances, appear to contact the substrate. Since scent marks originating from different sources may convey different messages (Johnson 1973), a single twist mark may convey at least two

messages. Rasa (1973) found that secretions from the anal glands of the mongoose serve to identify the individual while cheek gland secretions convey threat. A complete mark involves secretions from both glands. Muller-Schwarze (1971) also reported different functions for the scent from different glands in the black-tailed deer; the metatarsal gland scent was discharged in fear situations and the tarsal gland scent seemed important in individual recognition.

Rasa (1973) found sex differences in gland utilization; the male mongoose marks more frequently with anal glands than the female. In the groups from which marking data were collected regularly, male squirrels marked more frequently with the oral gland while females twist mark more often. If the oral and dorsal glands convey different messages, an explanation for these differences in gland utilization is offered. Because of the potential of the dorsal gland field for passive non-oriented burrow marking (as mentioned in the Gland Location and Scent Marking section), Steiner (personal communication) has suggested that the secretions from these glands may convey a message of burrow occupancy (i.e., "This burrow is occupied.").

If the secretions from the oral gland carried a "threat" message and secretions from the dorsal glands an "occupied" message, then a twist mark would carry both messages. It may be important for an adult female to convey that the single burrow in which she is rearing her young is occupied and to also issue a threat message; thus, females twist mark more readily. Murie (personal communication) recorded (field observation) a predominance of twist marking in female *C. columbianus* during the pregnancy-lactation period. On the other hand, a male does not mark, predominantly, the area surrounding the burrow in which he

usually spends the night. Unlike a female with young, a male has no reason to restrict his activities to a single burrow. Males appear to be more concerned with the entire territory; therefore, a threat or adult maleness message, which may be contained in oral gland secretions, might be more useful to a territorial male.

Anal Gland Scent Mark

If any mention is made of scent communication in the studies of ground squirrels, the anal glands are most commonly considered, perhaps because of the obvious pulsating movement they exhibit. Many investigators have associated this pulsating movement of the anal glands, which may indicate the release of secretions, with situations where the animal is involved in hostile encounters (e.g., *S. armatus*, Balph and Stokes (1973); *S. undulatus*, Gilmore (1934)) or is frightened or excited (e.g., *S. beechyi*, Evans and Holdenrold (1943)).

It is difficult to determine if the anal glands of *S. columbianus* are involved in active marking; however, Steiner (1974) reports that the anal glands in this species pulsate when an animal is frightened during hostile encounters. The pulsating movement of an everted anal gland indicates that a volatile substance is being released according to Hatt (1926). Since a volatile substance diffuses at a relatively high rate, any message contained in the volatile secretions would be rapidly conveyed. I have noticed an extremely pungent odor, different from oral gland secretions or urine trails, when handling an excited animal whose anal glands were pulsating. These data seem to point toward a "repelling" or "distress" signal being present in anal gland secretions.

Anal gland secretions may also be involved in anal drag marking (see

Scent Marking section for details). In adult males, anal drag marking is almost completely restricted to the breeding season (Steiner 1974). The apparent abscence of seasonal variations in secretory activity of the anal glands, combined with the urine-like appearance of secretions deposited during anal drag marking, suggests that the anal glands may not contribute to this type of marking. Large sebaceous glands in the prepuce region may add materials to these liquid secretions. In mice, lipid secretions of the male preputial gland have attractive properties (Caroom and Bronson 1971). Steiner (1974) also pointed out that "urine trails" of *S. columbianus* have attractive properties, possibly as a result of the presence of androgen metabolites. These metabolites might coincide with reproductive development in males.

INFLUENCES OF AN ANIMAL'S OWN SCENT ON HIMSELF

Besides conveying information to conspecifics, a scent mark may also affect an animal's own behavior. In this section I consider this possibility.

If the scent marks are distributed throughout a territory, not just at boundaries, then personal use of these marks are enhanced (Johnson 1973). Since both male and female *S. columbianus* mark throughout their territory, a scent mark might be of orientation value to the resident. It is important for an animal to make an appropriate behavioral response to stimuli; the nature of this response depends to some extent on the location of an animal with respect to its territory. For example, if an animal is near the periphery of his territory his flight threshold might be lower than when he is at a more central location in his territory.

Ewer (1968) feels that familiar odors in an animal's area provide "self-assurance" for the resident. In *S. columbianus* a "self-assurance" function is possible but if this were the only function of a scent mark, then one would have expected the subordinate male of the pair (see Scent Marking and Social Setting) to mark more frequently than the dominant. The subordinate, not the dominant, was obviously the squirrel that needed more "self-assurance."

Along somewhat the same lines, the odor field hypothesis proposed by Eisenberg and Kleiman (1972) (see Motivation and Control of Scent Marking section for description) is another manner in which a squirrel might utilize its own scent. In order to establish and maintain a certain level of familiar scent, a male holding a large territory would have to mark more frequently than a male with a small territory. This was observed (see Scent Marking and Territorial Behavior section). In the Scent Marking and Social Setting experiment, high levels of marking on the first day the male was introduced may have resulted from a propensity to establish familiar scent.

SCENT AND THE GROUP

The use of scent as a mode of communication is most obvious in adult males; however, scent may also play an integral role in communication among other members within a group. Scent marking is performed by adult males and to a lesser extent by adult females, but greeting (see Annual Cycle for details), which also involves secretions from the oral gland, is performed by all group members. The involvement of scent within the group is discussed here.

Recognition of other group members seems essential for maintenance

of group integrity. Reciprocal investigation of oral glands (greeting) when two animals meet suggests individual identification or discrimination at a less precise level. Greeting may increase cohesiveness and/or stability of the group. Steiner (1974) states that both Arctic and Columbian ground squirrels apparently identify individuals and/or group members by scent. Recognition of a group scent was found in the sugar glider (Schultze-Westrum 1970) and the common marmoset (Epple 1970). In Richardson ground squirrels, Sheppard and Yoshida (1971) and Michener (1974) found that individuals discriminated between related and unrelated individuals, probably through olfaction.

On the group level a system of scent communication where individuality and nothing else is conveyed during greeting may not be adequate to explain the role of scent in the social system of Columbian ground squirrels. The ability to recognize a specific scent for four categories of animals (adult males, adult females, yearlings, and juveniles) plus a group scent would be sufficient to explain the behavior I observed. Of course, individual discrimination plus these categories or individual discrimination plus group scent recognition might also be functional.

In this social system, high levels of greeting among group members that occur during the first three or four weeks of the active season may result in enough scent exchange (scent sharing) to produce a group scent that is recognizable by all group members. Since the resident male initiates a larger percent of the greeting behavior, his scent would probably dominate such a scent pool (Steiner, personal communication).

Because of the group scent, intergroup movement by females and yearlings would be possible for a short interval early in the year until

they are "branded" with a group scent. I did not observe much movement from group to group early in the active season, but I did observe a resident male greet and then chase a pregnant female from a neighboring group out of his territory. This female may have acquired the scent of her own group by this time (two weeks into the active season) and was thus rejected.

In general, group composition was relatively stable throughout most of the active season with the exception of movements of yearling males after aggression displayed toward them by adult males and, to a lesser degree, by adult females. Yearling males were tolerated within the group until they exhibited some reproductive development approximately two weeks after peak adult male breeding activity. As in the adult males, reproductive development of yearling males is likely associated with hormonal changes which may influence the secretory activity of the integumentary glands. Because aggression between yearling and adult males was usually preceded by greeting, the yearling male may have developed an "adult male" scent. Adult males would then recognize yearling males as potential threats to their territory and attempt to exclude them. If only individuality were conveyed during greeting, an adult would be unable to detect this change during greeting. Of course, behavioral change or scent from other body regions could relate these changes in yearling males to adult males. However, olfactory investigation of body regions other than the oral gland region are infrequent after the breeding season.

When scent marking, an adult male would convey the same message as during greeting, "adult maleness." This single component could convey

threat and territorial ownership or an occupancy message to other adult males, and thus be functional in territorial behavior or convey to a female that she is in the territory held by a male with which she shares a common scent or scent pool. The meaning of a scent is, of course, dependent upon the recipient.

Juveniles and yearlings could also utilize scent marks of the resident male for orientation. The question arises: are the females selecting a burrow within a male's territory or are the males acquiring a territory that encompasses two or three females? In the former case, scent marks may attract the female and in the latter case, keep the females from leaving if it is important for a female to inhabit a territory occupied by an adult male. I did record an instance of a pregnant female moving to another group after the adult male was removed; thus, females may prefer an area in which an adult male is present.

A message of just individual identity in a scent mark seems sufficient to explain the involvement of marking in territorial behavior of males, but a message of only individuality seems inadequate when communication is considered on the group level.

SCENT AND OTHER COMMUNICATION MODALITIES

The importance of a certain communication modality to a species depends on characteristics of the habitat a species occupies and the degree of sociality of the species. For example, in general a species that occupies a terrestrial habitat where features of the terrain make vision and audition ineffective, or if it is highly social, might be expected to utilize olfaction more extensively than a species found in a habitat that does not restrict sight or sound or is a solitary animal.

"Scent glands will generally have signalling qualities when other sensory systems are depressed or otherwise nonfunctional" (Thiessen, Lindzey, Blum, and Wallace 1970:512). These authors also point out that scent communication will be enhanced when it is used along with other communication modalities. Integration of olfaction with other modes of communication used by *S. columbianus* is considered in this section.

Although visual and acoustic signals are not obscured to any great extent in the habitat occupied by Columbian ground squirrels, this species uses olfaction extensively, possibly because of their complex social system. Scent may permit recognition to an extent that the social group can be maintained. High levels of greeting early in the season when social groups are being established may enhance development of a group scent and recognition of individuality and/or status. Visual cues could and may replace olfaction to some extent later in the season when group members learn where to expect other group members and to recognize behavioral responses of these animals; thus greeting is less frequent at this time.

Of course, durability of a scent mark may augment the effectiveness of visual signals. For example, after detecting a scent mark an intruding male's behavior may be more easily modified or directed into making a correct choice (i.e., to flee or fight) when a visual cue, such as the appearance of a resident male, is later added to a scent cue.

Scent marks may also enhance visual cues used by an individual for location in relation to their territory. Thus visual and auditory senses would be freed for detection of predators. When an alarm call is given most squirrels appear to know immediately where the nearest burrow

is located, especially when they are within their group territory.

Even though Columbian ground squirrels are diurnal the dark burrow system in which they spend a good deal of time is unsuitable for visual communication. Acoustic signals could and likely do provide some communication, but again, olfaction has more potential for recognition of group members in the dark confines of a burrow.

Scent marking may also have a visual component. Postures assumed during marking, especially twist marking, may serve to attract another squirrel to a mark. Since adult males and to a lesser degree adult females perform most marking and may utilize oral and twist marking differentially (see Scent Marking section), age and sex of an individual might be visually conveyed.

CONCLUSIONS

As pointed out by Eisenberg and Kleiman (1972), scent should be considered as a means of orientation, information exchange, and integrating reproductive and social behavior, not just as a means of territorial defense. Scent in Columbian ground squirrels is apparently involved to some extent in territorial behavior but also seems to play an important role in the social behavior of this species.

Secretory activity of integumentary glands in both males and females peaked during intervals when highest levels of scent marking were also recorded, the breeding season for males and the pregnancy-lactation period for females. Because territorial behavior is most vigorous when marking is high, an involvement of scent marking in territoriality is suggested. At least in adult males, testosterone likely influences secretory activity of integumentary glands and to some extent marking behavior. Hormones associated with pregnancy and lactation may serve this function in females, although this possibility was not explored in this study.

Scent marks may serve several functions. A scent mark does not repel but does appear to lower the flight threshold of an intruder. Because scent marks are distributed throughout a territory, the scent may be for an animal's own use, to familiarize his surroundings or for orientation purposes. Other group members may also utilize their own scent marks or those deposited by a resident male.

Both field observations and controlled pen experiments indicated that the presence of a reproductively active female may elicit increased

marking by males and increased male-male aggression. It is possible that male scent marks attract females or convey that an adult male holds the territory they are occupying. Whatever the message, since the presence of adult females in a male's territory enhances his reproductive fitness, it seems likely that message(s) contained in a scent mark, deposited by an adult male, may directly or indirectly influence the females' behavior.

Adult males apparently can discriminate between their own scent mark and scent marks of strange males. The scent discrimination abilities of this species were not investigated further but this ability, on some level, seems essential to explain the obvious implications of scent in the group. Discrimination of five categories of animals (adult males and females, yearling males and females, and juveniles) probably occurring during greeting, plus a scent common to all group members would be adequate. Of course, this system would be functional if individual recognition existed in addition to the five categories, or individual plus group scent recognition, but not with individual recognition alone.

In brief, olfaction appears to be important and perhaps essential for maintenance of a stable group integrity. Besides enhancing communication based on other modalities, scent may provide a means of recognition that allows a social group to be formed and then maintained throughout the active season. Because a social group persists after the breeding season, adult females, some yearlings, and later juveniles likely benefit from the resident male's territorial behavior. The aggressiveness toward intruders and alertness of a resident male may provide added protection for other group members that would not be present if the social group did not exist. Armitage (1974) felt that territoriality in

marmots enhanced fitness of a colonial male. This also seems plausible for male *S. columbianus*.

Table 1. Comparison of oral (a) and dorsal (b) gland dimensions of adult male and female Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different. All measurements are in millimeters.

	N	Male	N	Female
Oral Gland (a)				
Dorsal-Ventral	44	2.21 ± 0.06	48	1.88 ± 0.06
Lateral	44	1.51 ± 0.06	48	1.09 ± 0.05
Anterior-Posterior	43	3.24 ± 0.09	46	2.78 ± 0.07
Dorsal Gland (b)				
Dorsal-Ventral	37	0.32 ± 0.01	40	0.21 ± 0.09
Lateral	37	0.72 ± 0.03	40	0.46 ± 0.02
Anterior-Posterior	37	0.88 ± 0.03	40	0.61 ± 0.02

Table 2. Macroscopic comparison of dorsal gland dimensions of adult male and female Columbian ground squirrels ($\bar{X} \pm S.E.$). Values underlined at the same level are not significantly different. All measurements are in millimeters.

	N	Male	N	Female
Dorsal Glands				
Lateral	17	0.83 ± 0.08	20	0.41 ± 0.04
Anterior-Posterior	17	1.14 ± 0.10	20	0.67 ± 0.05
Gland Field				
Anterior-Posterior	13	99.08 ± 5.42	9	96.67 ± 5.77
Lateral	14	42.00 ± 0.86	8	43.75 ± 2.06
Dorsal Glands Ventral Ear				
Lateral	16	1.62 ± 0.14	19	1.10 ± 0.10
Anterior-Posterior	16	2.02 ± 0.15	19	1.44 ± 0.11
Dorsal Glands				
Total No. of Glands	6	56.67 ± 6.54	4	62.50 ± 8.54
No. of Glands Ventral Ear	6	9.16 ± 0.48	4	9.50 ± 0.64

Table 3. Comparison of dorsal glands (ventral ear) charcteristics of adult male and female Columbian ground squirrels ($\bar{X}$ ± S.E.). Values underlined at the same level are not significantly different.

	N	Male	N	Female
Tubule Diameter (μ)	5	135.0 ± 13.5	4	57.5 ± 6.3
Sinus Diameter (μ)	5	236.0 ± 25.0	4	86.3 ± 4.7
Epithelium Height (μ)	5	24.2 ± 1.6	4	15.4 ± 2.2
Epithelium Height (Sinus) (μ)	5	15.7 ± 0.9	4	8.0 ± 1.4
Activity Index (%)	5	76.2 ± 7.3	4	44.0 ± 9.7
Dorsal-Ventral (mm)	5	0.75 ± 0.1	4	0.44 ± 0.1
Lateral (mm)	5	1.34 ± 0.1	4	0.68 ± 0.1
Anterior-Posterior (mm)	5	1.92 ± 0.3	4	1.16 ± 0.2
Sebaceous gl. Diameter (μ)	5	49.00 ± 7.0	4	32.00 ± 2.9

Table 4. Comparison of oral (a) and dorsal (b) gland characteristics of yearling male and female Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	N	Male	N	Female
Oral Gland (a)				
Tubule Diameter (μ)	9	65.20 ± 2.00	5	50.67 ± 3.47
Epithelium Height (μ)	9	9.50 ± 0.33	5	9.40 ± 0.48
Activity Index (%)	9	57.20 ± 6.19	5	49.33 ± 12.09
Dorsal-Ventral (mm)	9	2.17 ± 0.10	5	1.90 ± 0.14
Lateral (mm)	9	1.34 ± 0.10	5	1.00 ± 2.42
Anterior-Posterior (mm)	9	3.11 ± 0.10	5	2.93 ± 0.20
Dorsal Gland (b)				
Tubule Diameter (μ)	1	66.0	1	80.0
Epithelium Height (μ)	1	19.9	1	20.0
Activity Index (%)	1	64.3	1	68.2
Dorsal-Ventral (mm)	1	0.26	1	0.31
Lateral (mm)	1	0.55	1	0.53
Anterior-Posterior (mm)	1	0.60	1	0.80

Table 5. Comparison of oral gland characteristics of adult male Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	Breeding	Postbreeding
N	21	23
Tubule Diameter (μ)	71.5 ± 1.4	69.1 ± 1.2
Epithelium Height (μ)	10.7 ± 0.3	8.6 ± 0.2
Secretory Activity Index (%)	66.9 ± 5.0	36.8 ± 3.3
Sebaceous Gland Diameter Duct Region (μ)	82.7 ± 2.2	74.2 ± 1.0
Sebaceous Gland Diameter Post-duct Region	21.2 ± 0.9	20.0 ± 0.4

Table 6. Comparison of oral gland characteristics of adult female Columbian ground squirrels ($\bar{X}$ ± S.E.). Values underlined at the same level are not significantly different.

	Breeding	Pregnant	Lactating	Post Lactating
N	13	11	13	11
Tubule Diameter (μ)	49.0 ± 2.0	52.2 ± 1.8	56.3 ± 1.8	56.5 ± 2.6
Epithelium Height (μ)	9.5 ± 0.2	9.3 ± 0.3	9.6 ± 0.4	9.6 ± 0.4
Secretory Activity Index (%)	25.0 ± 3.5	29.2 ± 3.4	55.0 ± 4.9	31.4 ± 4.7
Sebaceous Gland Diameter Duct Region (μ)	37.3 ± 2.0	56.7 ± 1.8	59.9 ± 1.1	63.1 ± 1.0
Sebaceous Gland Diameter Post-duct Region (μ)	23.5 ± 1.0	22.2 ± 1.0	21.3 ± 0.4	21.8 ± 0.6

Table 7. Comparison of dorsal gland characteristics of adult male Columbian ground squirrels ($\bar{X}$ ± S.E.). Values underlined at the same level are not significantly different.

	Breeding	Postbreeding
N	17	20
Tubule Diameter (μ)	92.9 ± 2.6	84.5 ± 2.5
Epithelium Height (μ)	20.3 ± 0.8	16.0 ± 1.1
Secretory Activity Index (%)	84.6 ± 3.4	42.9 ± 4.6
Sebaceous Gland Diameter (μ)	46.5 ± 1.9	30.2 ± 1.5

Table 8. Comparison of dorsal gland characteristics of adult female Columbian ground squirrels ($\bar{X}$ ± S.E.). Values underlined at the same level are not significantly different.

	Breeding	Pregnant	Lactating	Post Lactating
N	11	9	11	9
Tubule Diameter (μ)	48.5 ± 2.2	58.0 ± 3.4	56.2 ± 3.3	63.8 ± 1.8
Epithelium Height (μ)	12.5 ± 1.2	14.6 ± 0.8	12.5 ± 1.2	12.3 ± 0.9
Secretory Activity Index (%)	27.7 ± 3.4	47.6 ± 6.2	41.5 ± 4.3	30.8 ± 5.4
Sebaceous Gland Diameter (μ)	30.3 ± 1.7	35.7 ± 2.7	25.3 ± 1.3	25.2 ± 1.2

Table 9. Comparison of anal/gland characteristics (apocrine portion) of adult male Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	Breeding	Postbreeding
N	11	10
Tubule Diameter (μ)	39.6 ± 2.7	36.9 ± 1.9
Epithelium Height (μ)	13.1 ± 0.5	10.0 ± 0.4
Secretory Activity Index (%)	47.5 ± 6.5	33.1 ± 3.5

Table 10. Comparison of anal gland characteristics (apocrine portion) of adult female Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	Breeding	Postbreeding
N	9	12
Tubule Diameter (μ)	39.1 ± 0.9	41.5 ± 0.8
Epithelium Height (μ)	10.9 ± 0.5	13.4 ± 0.6
Secretory Activity Index (%)	31.0 ± 5.0	46.8 ± 3.8

Table 11. Comparison of oral gland characteristics of castrated, castrated and testosterone treated, and intact adult male Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	Castrated Testosterone	Castrated	Intact Control
N	5	5	4
Tubule Diameter (μ)	72.2 ± 1.2	77.0 ± 0.7	68.8 ± 1.5
Epithelium Height (μ)	10.4 ± 0.6	7.7 ± 0.4	7.4 ± 0.1
Secretory Activity Index (%)	55.3 ± 4.3	22.7 ± 4.3	14.0 ± 0.6
Sebaceous Gland Diameter (μ)	93.8 ± 7.0	60.8 ± 3.0	51.0 ± 3.5

Table 12. Comparison of dorsal gland characteristics of castrated, castrated and testosterone treated, and intact adult male Columbian ground squirrels ($\bar{X} \pm$ S.E.). Values underlined at the same level are not significantly different.

	Castrated Testosterone	Castrated	Intact Control
N	5	5	4
Tubule Diameter (μ)	95.6 ± 1.6	79.4 ± 3.2	88.8 ± 2.1
Epithelium Height (μ)	22.0 ± 1.2	18.5 ± 0.4	19.4 ± 1.1
Secretory Activity Index (%)	92.9 ± 1.8	81.6 ± 3.9	67.9 ± 4.3
Sebaceous Gland Diameter (μ)	64.0 ± 2.5	42.4 ± 1.2	39.0 ± 2.2

Table 13. Comparison of marking frequency of dominant and subordinate male Columbian ground squirrels before and after reversals in dominance after testosterone was administered to the original subordinate. Marking frequency (pegs and ground) of both the original dominant and subordinate differed significantly between the two test periods ($\bar{X} \pm$ S.E.).

	Before Reversal		After Reversal	
	Mean Marking Frequency/Hour			
	Pegs	Ground	Pegs	Ground
Original Dominant	0.85 ± 0.09	2.23 ± 0.49	0.06 ± 0.06	1.61 ± 0.58
Original Subordinate	0.47 ± 0.09	1.90 ± 0.55	1.70 ± 0.31	6.28 ± 1.74

Table 14. Influence of social setting (Combinations A, B, and C) on frequencies of marking and aggressive encounters for resident and nonresident males plus frequency of clawing for resident males ($\bar{X}$ ± S.E.). Each value is a mean for a three-day interval. Values underlined at the same level are not significantly different.

Combination	Days 1-3	4-6	7-9
	Resident Marking Frequency		
A	19.1 ± 4.8	27.3 ± 6.5	27.9 ± 5.7
B	19.7 ± 4.9	30.1 ± 7.7	-
C	20.3 ± 4.3	23.8 ± 4.8	-
	Nonresident Marking Frequency		
A	-	4.5 ± 1.4	5.8 ± 2.5
B	-	5.7 ± 2.1	-
	Male-Male Aggressive Encounters		
A	-	10.9 ± 2.4	11.7 ± 3.6
C	-	9.6 ± 1.8	-
	Resident Clawing		
A	1.7 ± 0.8	2.3 ± 1.0	4.2 ± 1.4
B	1.5 ± 0.8	3.7 ± 1.4	-
C	2.4 ± 1.1	3.3 ± 1.2	-

Table 15. Influences of differing scent stimuli on marking. Total number of times each peg (Stranger, Own, Neutral) was marked during each interval and total plus the Chi Square Values and probability are presented. Data collected for the subject during breeding and postbreeding periods are expressed separately.

Interval	Stranger	Own	Neutral	χ^2 Value	Probability
		Breeding			
1st	86	58	65	6.10	P<.05
2nd	46	30	26	6.59	P<.05
3rd	32	15	17	8.09	P<.025
4th	20	13	10	3.67	N.S.
3rd+4th	52	28	27	11.23	P<.005
Total	184	116	118	21.50	P<.005
		Postbreeding			
1st	60	67	47	3.55	N.S.
2nd	14	7	7	3.50	N.S.
3rd	15	3	1	18.11	P<.005
4th	2	1	1	0.50	N.S.
3rd+4th	17	4	2	17.31	P<.005
Total	91	78	56	8.35	P<.025

Table 16. Influence of differing scent stimuli on marking. Comparison of breeding and postbreeding values (see Table 15) from equivalent five minute intervals and totals. The Chi Square value and probability are presented for each interval.

Interval	Stranger		Own		Neutral	
	X^2	P	X^2	P	X^2	P
1st	4.28	<.05	0.51	N.S.	2.58	N.S.
2nd	16.02	<.005	13.08	<.005	9.81	<.005
3rd	5.45	<.025	6.72	<.01	12.50	<.005
4th	13.14	<.005	8.64	<.01	5.82	<.025
3rd+4th	16.75	<.005	16.50	<.005	19.86	<.005
Total	30.78	<.005	7.06	<.01	21.39	<.005

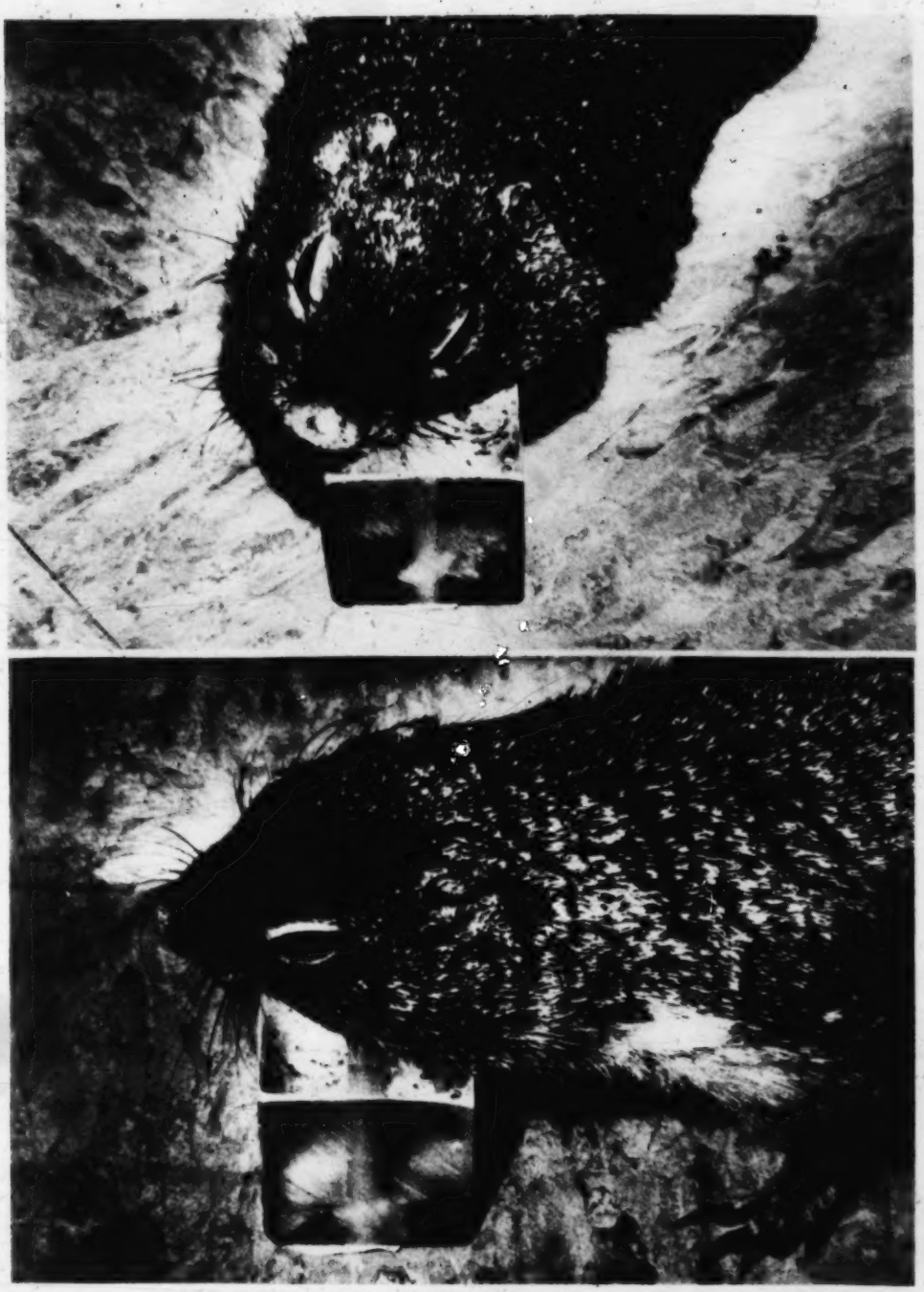


Fig. 1. Columbian ground squirrel marking a marking peg with the oral gland front view (top) and side view (bottom).

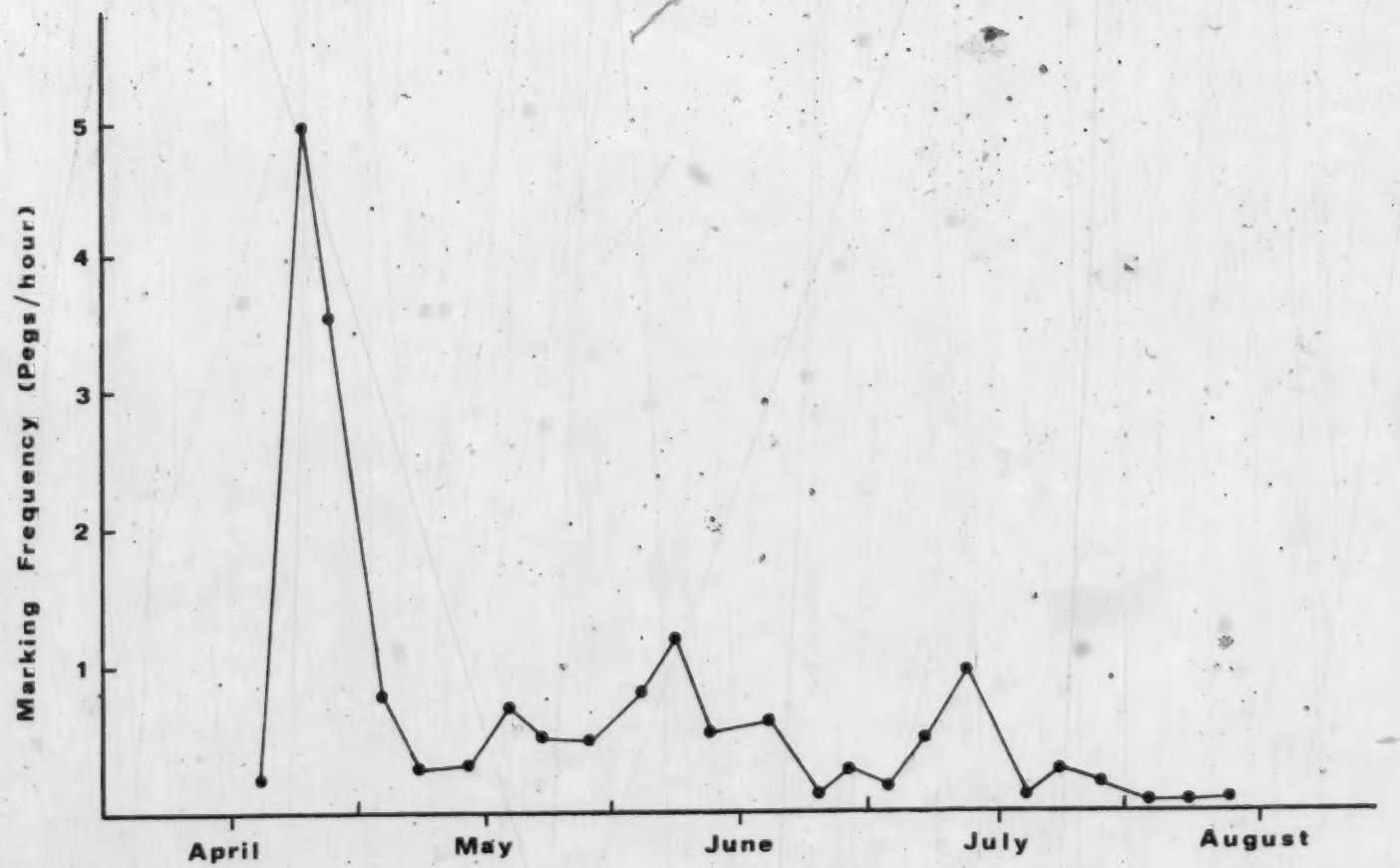
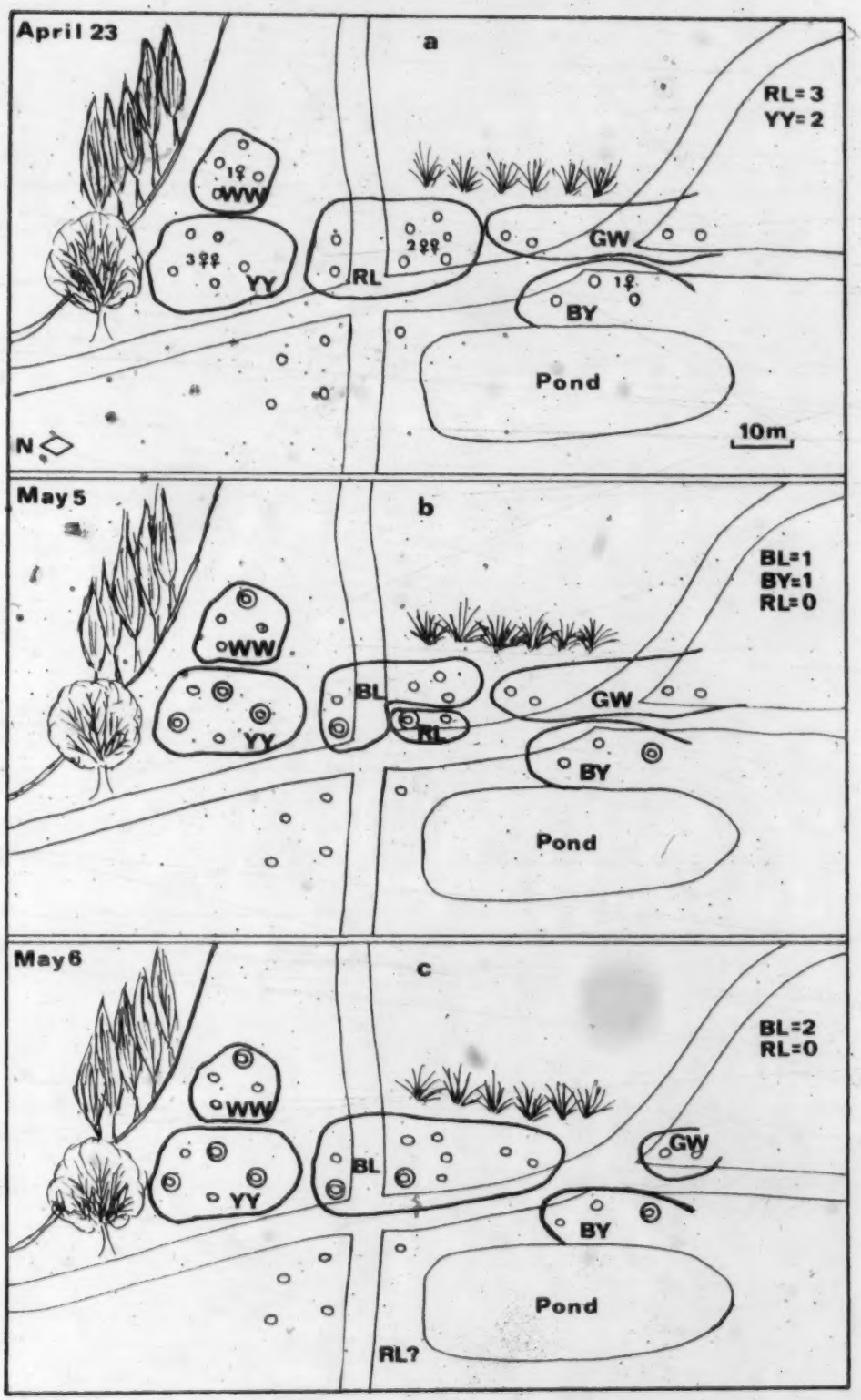


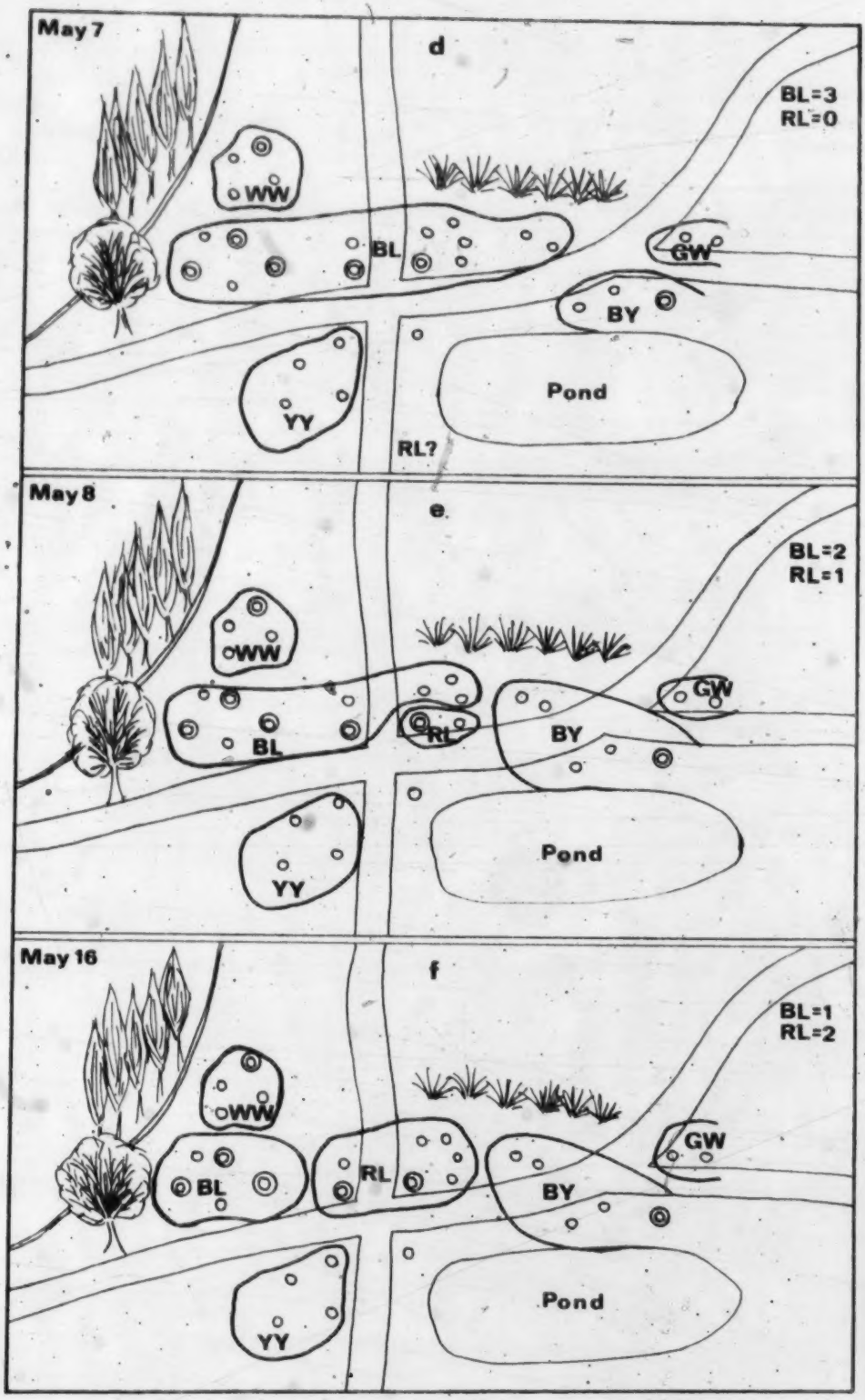
Fig. 2. Seasonal variations in frequency of scent marking with the oral gland for adult male Columbian ground squirrels during the active season. Each point represents a mean value for data collected during a five day interval, minimum of three days data.

Fig. 3. Changes in territory ownership during the spring of 1973. Marking frequency in pegs/ hour, when available on that date (upper left), is presented on the upper right. Each territory is outlined by a heavy line with the male squirrel (RL, BL, etc.) holding that territory lettered inside. The number of females that emerged from hibernation within each territory is shown in figure (a).

Major burrows = o

Burrows occupied by adult females = ◎


April 23
a
RL=3
YY=2
1♀
WW
3♀♀
YY
2♀♀
RL
GW
1♀
BY
Pond
N
10m
May 5
b
BL=1
BY=1
RL=0
WW
YY
BL
RL
GW
BY
Pond
May 6
c
BL=2
RL=0
WW
YY
BL
GW
BY
Pond
RL?


May 7
d
BL=3
RL=0
WW
BL
GW
BY
YY
Pond
RL?
May 8
e
BL=2
RL=1
WW
BL
RL
GW
BY
YY
Pond
May 16
f
BL=1
RL=2
WW
BL
RL
GW
BY
YY
Pond

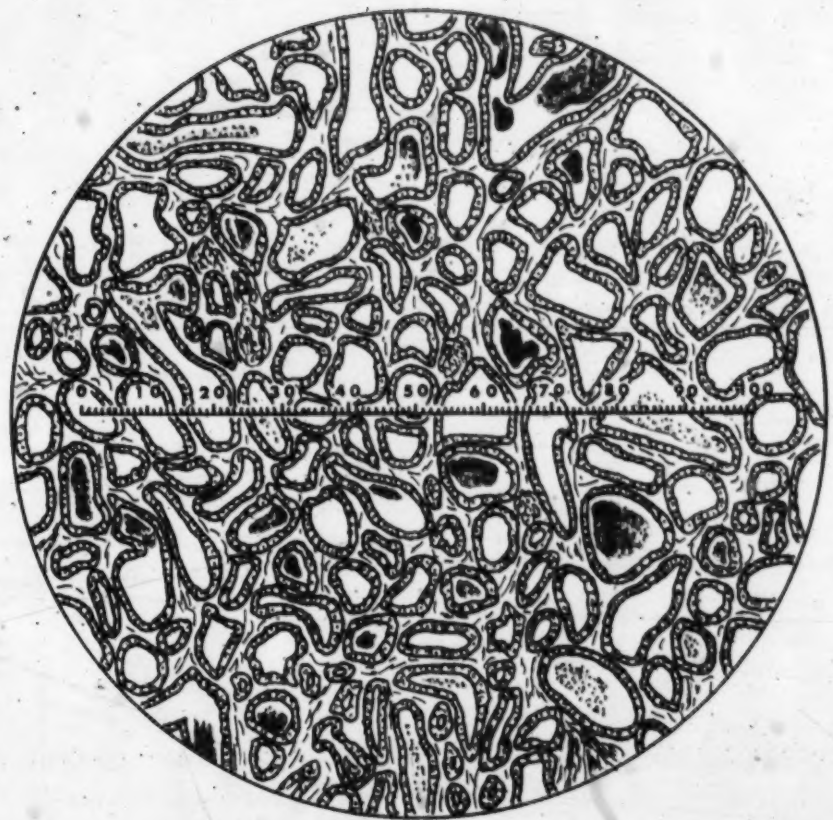


Fig. 4. Oral gland tissue as viewed through a light microscope showing the micrometer that was used as a transect (0-10 on micrometer=100μ).

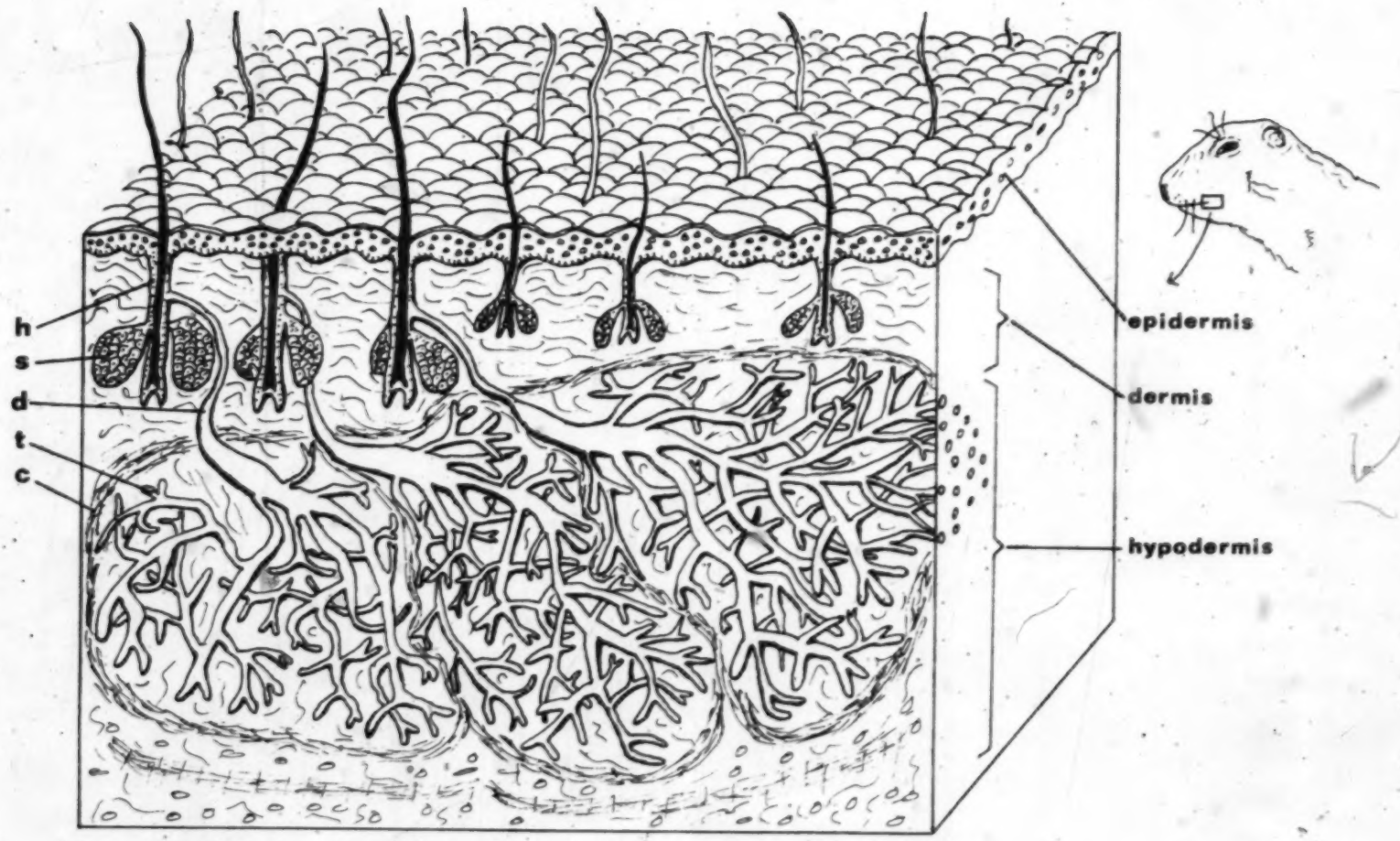


Fig. 5. Section of skin from the oral angle region showing the branched tubule structure of apocrine gland tissue. h = hair follicle s = sebaceous gland d = duct from apocrine gland t = apocrine gland tubule c = connective tissue.

Fig. 6. Cytoplasmic protrusions (budding) on the luminal surface of an epithelial cell (oral gland) of *S. columbianus* (a), Myoepithelial cell lying inside the basement membrane at the base of the secretory epithelium (b), duct from the apocrine portion of an oral gland emptying into a hair follicle (c), duct from an individual dorsal gland contacting the skin surface (d), and a duct originating from the sinus of a dorsal gland (e).

a
8μ
b
6μ
c
70
d
100 μ
e

Fig. 7. Oral gland of a postbreeding male showing adjacent actively secreting (right) and inactive portions (left). Note the difference in epithelial height.

Fig. 8. Section of an anal gland showing the apocrine portion (a), the sebaceous gland portion (b), and the retractable papillae channel (c).

30 µ

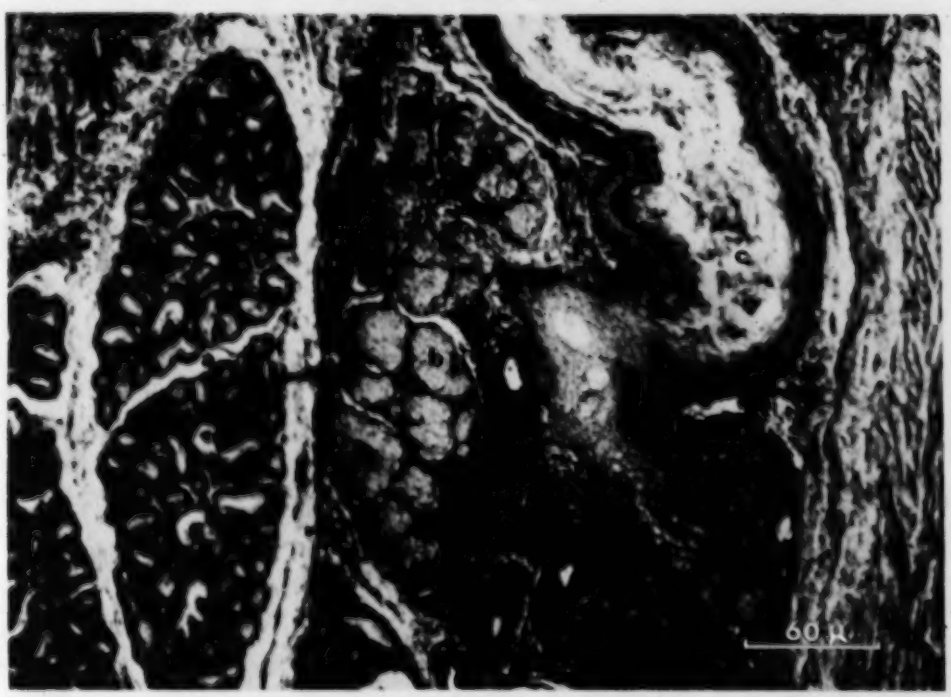
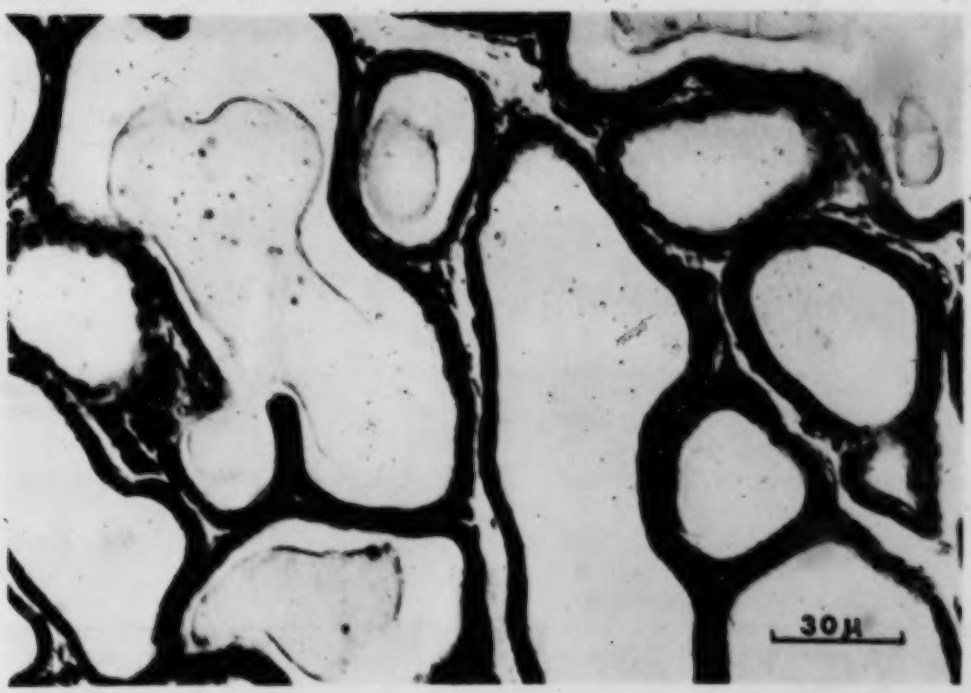

60 µ

Fig. 9. Inactive oral gland of a postbreeding male. Note the low epithelium and relative absence of secretory products in the tubules.

Fig. 10.. Active oral gland of a breeding male. Note the tall epithelium and abundance of secretory products in the tubules.


30μ

30μ

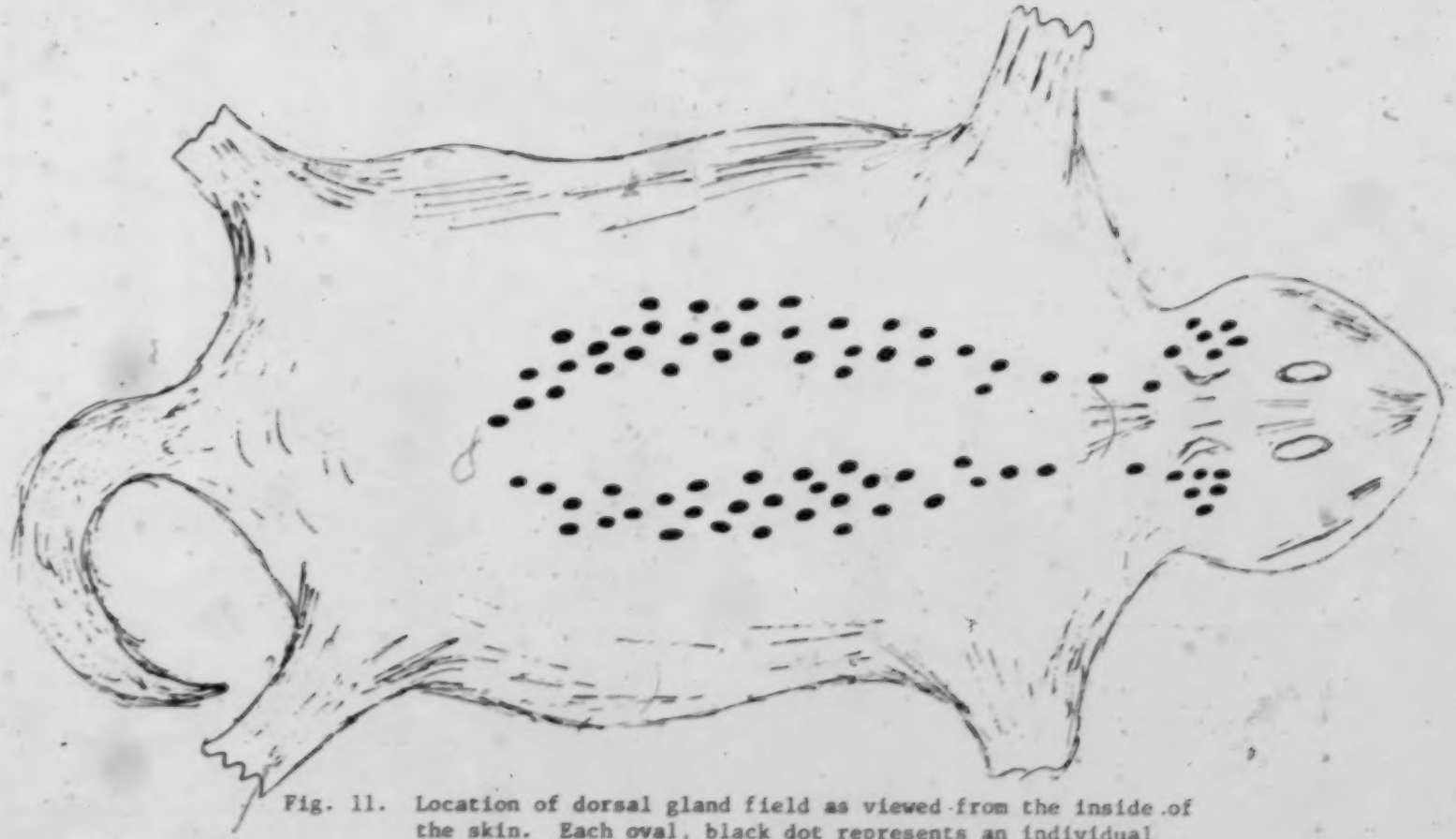
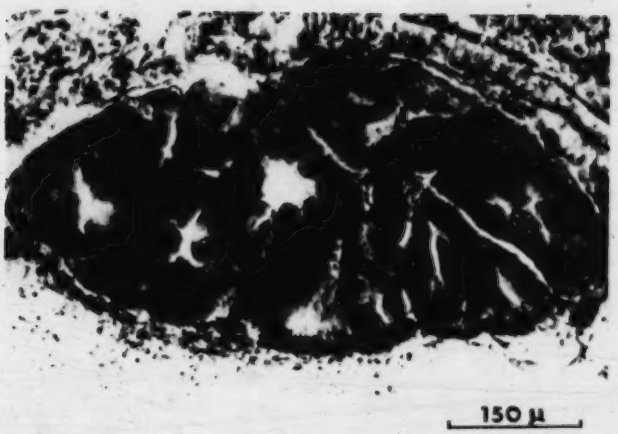


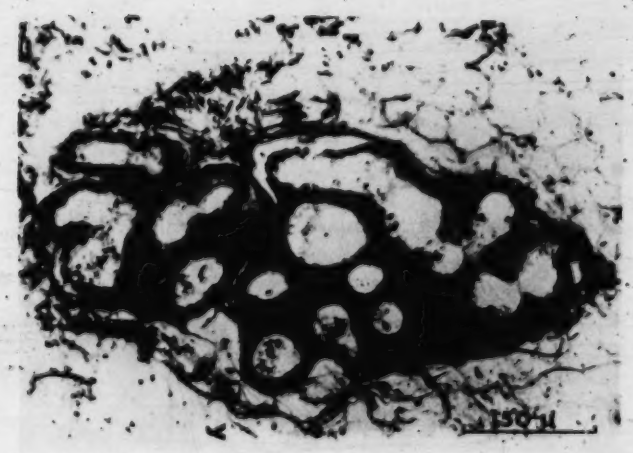
Fig. 11. Location of dorsal gland field as viewed from the inside of the skin. Each oval, black dot represents an individual dorsal gland.

Fig. 12. Sections through entire dorsal glands of adult male Columbian ground squirrels. Note the tall epithelium typical of early breeding animals (a), the tall epithelium and secretory products in the tubules in breeding males (b), and low epithelium of a spent gland of a postbreeding male (c).



a

b

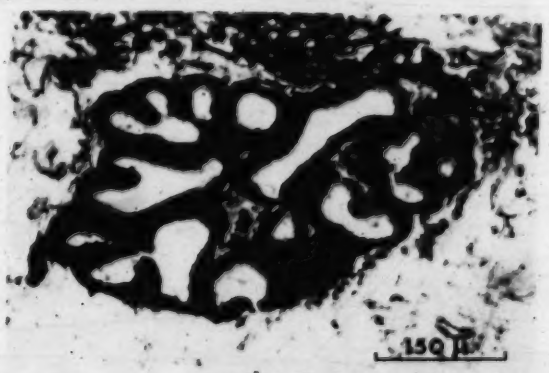


c

Fig. 13. Sections through entire dorsal glands of breeding (a), pregnant (b), lactating (c), and postlactating (d) female Columbian ground squirrels. Note the tall epithelium and presence of secretory products in the tubule lumens of (b) and (c).

b

c



d

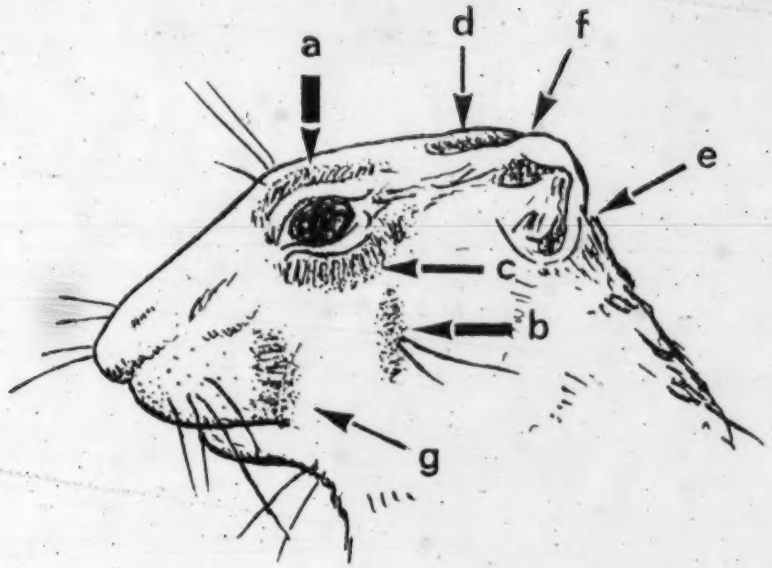


Fig. 14. Squirrel head reproduced from Steiner (1973) Can. J. Zool. 51:p158. All integument regions indicated by arrows were examined histologically.

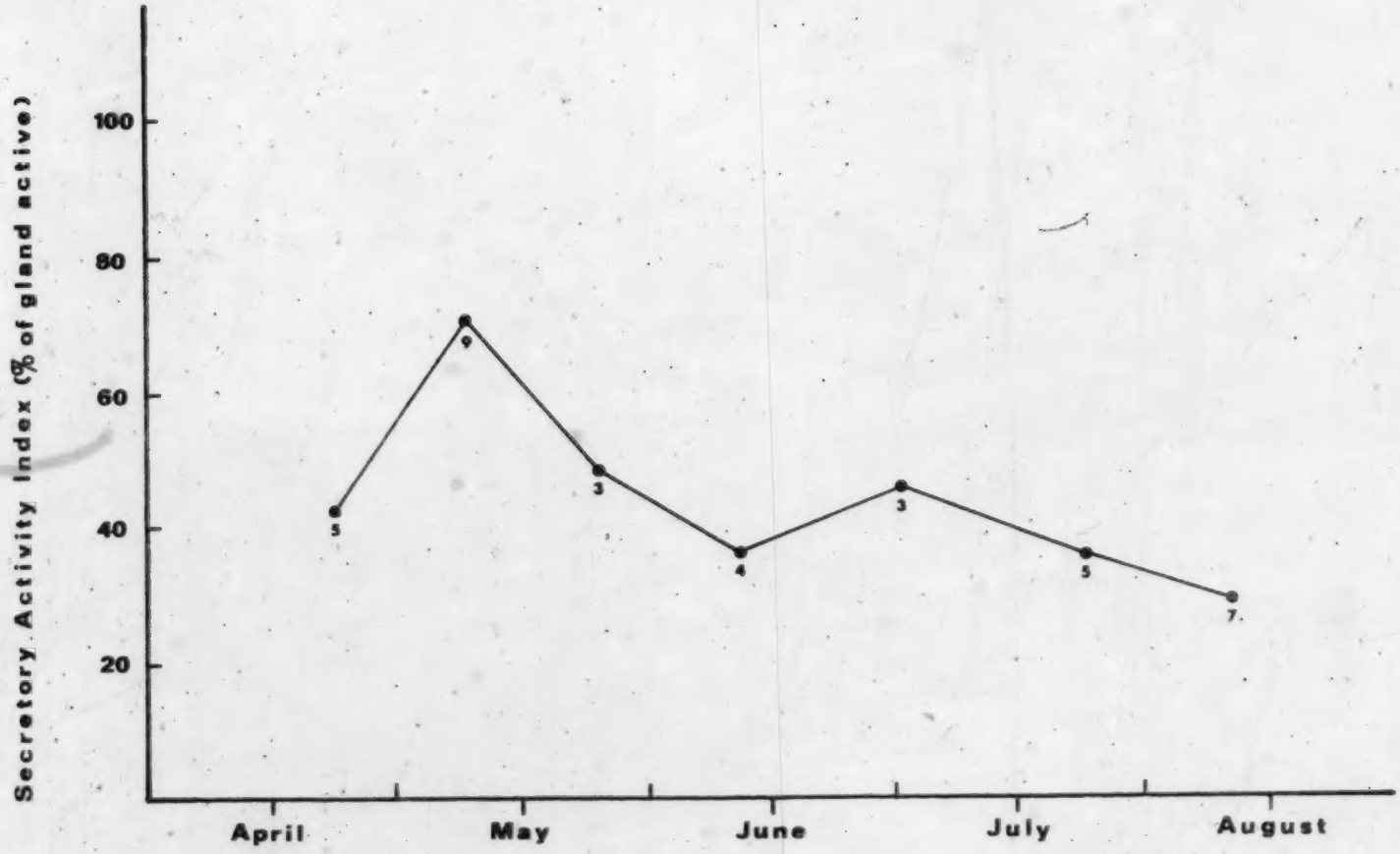
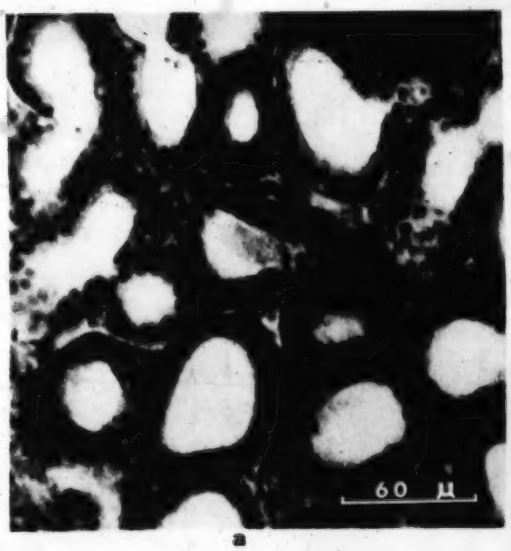


Fig. 15. Seasonal variations in secretory activity of the oral gland of adult male Columbian ground squirrels during the active season. N is presented below each point.

Fig. 16. Oral gland of breeding (a), pregnant (b), lactating (c), and postlactating (d) female Columbian ground squirrels. Tall epithelial cells and presence of secretory products in the tubule lumens (c) is indicative of intense secretory activity during lactation.



a

b

c

d

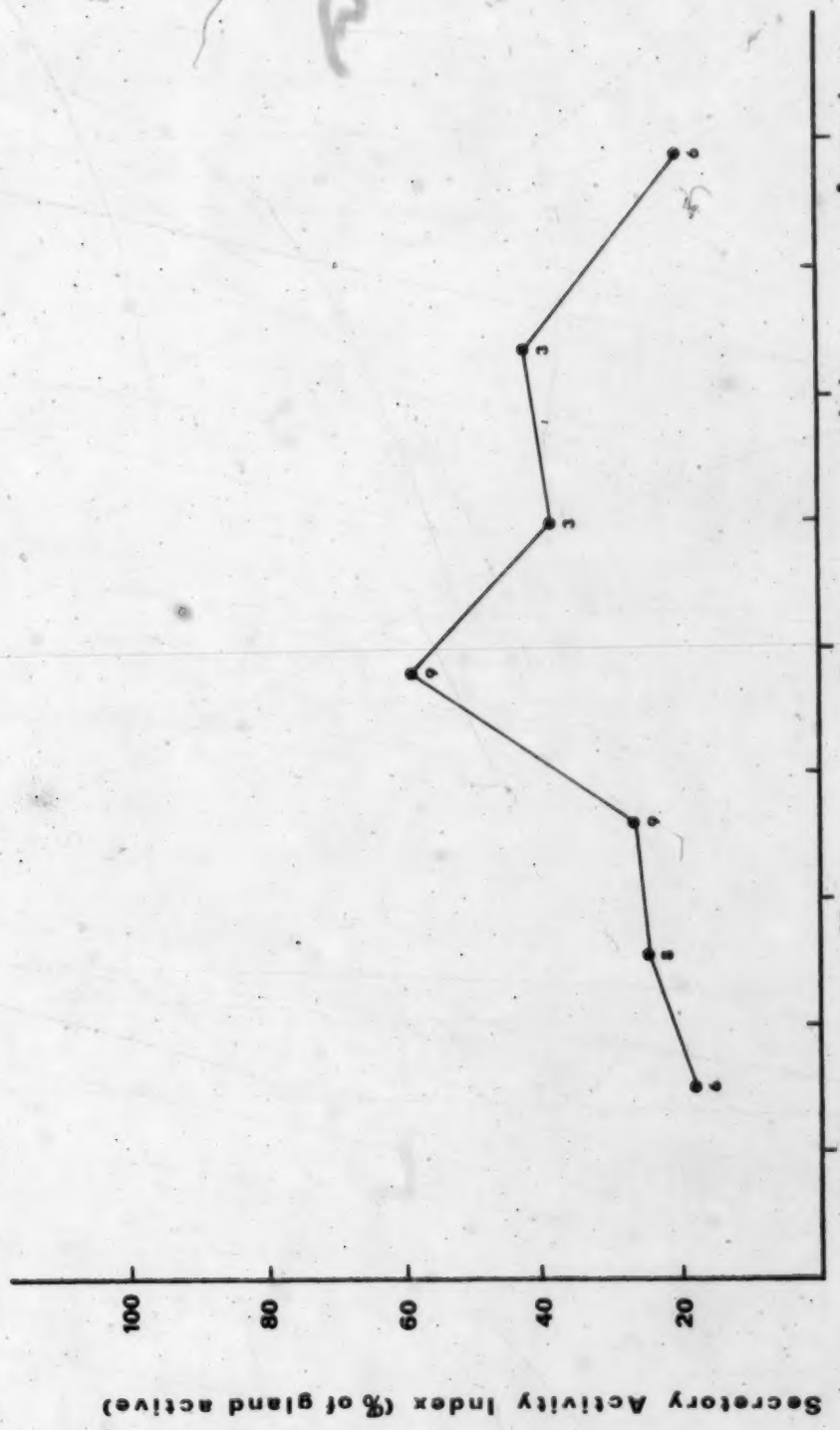


Fig. 17. Seasonal variations in secretory activity of the oral gland of adult female Columbian ground squirrels during the active season. N is presented below each point.

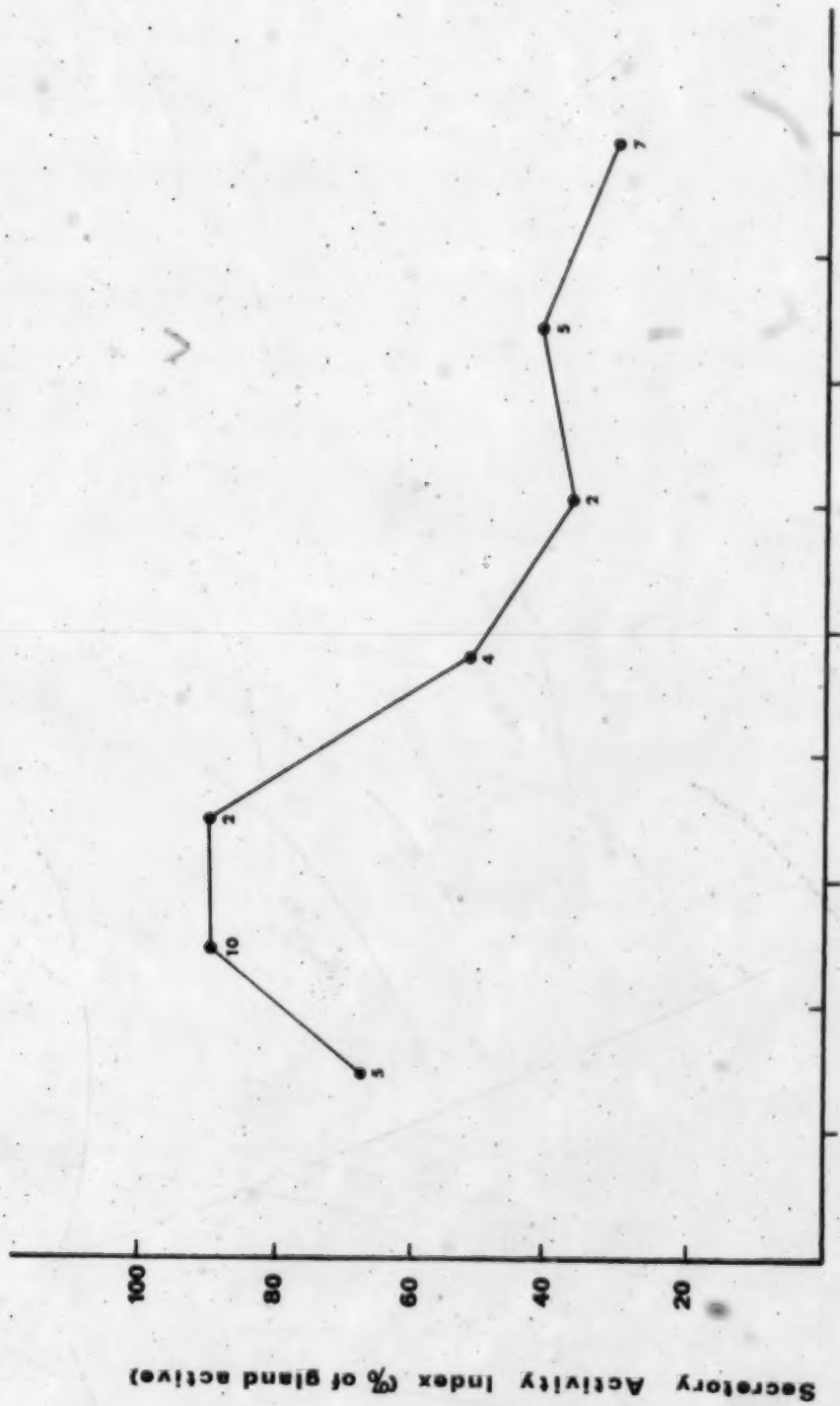


Fig. 18. Seasonal variations in secretory activity of the dorsal glands of adult male Columbian ground squirrels during the active season. N is presented below each point.

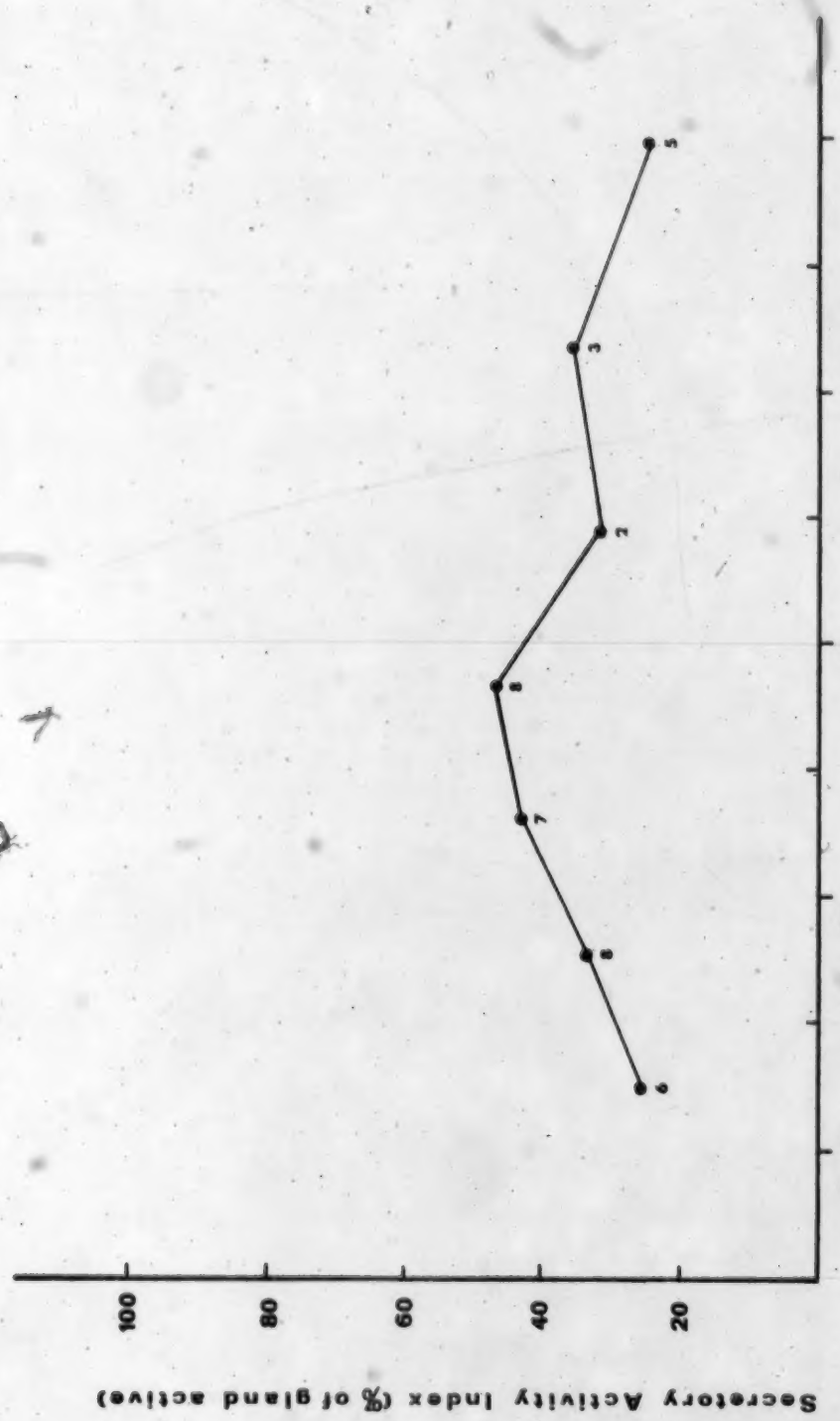


Fig. 19. Seasonal variations in secretory activity of the dorsal glands of adult female Columbian ground squirrels during the active season. N is presented below each point.

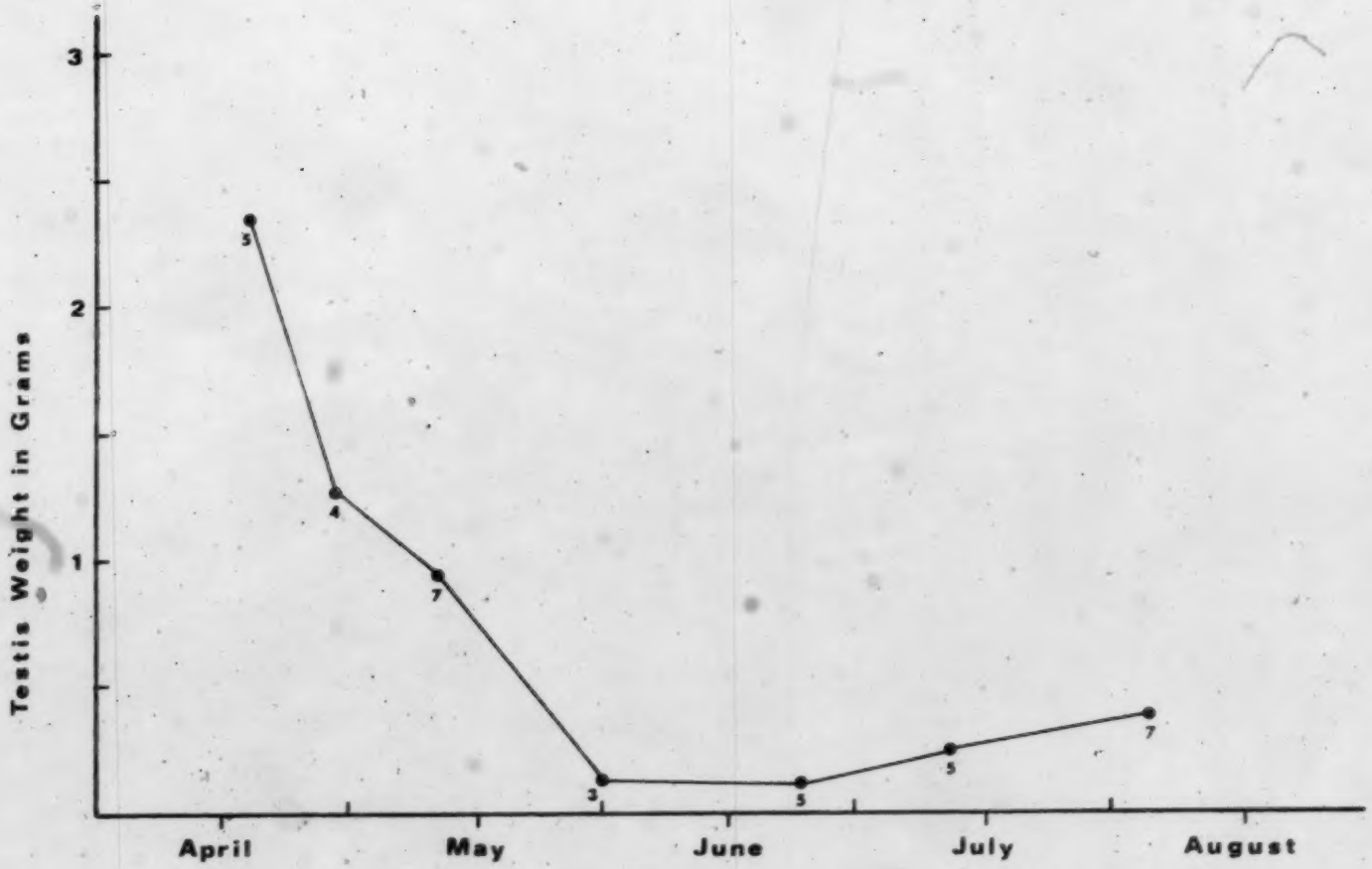
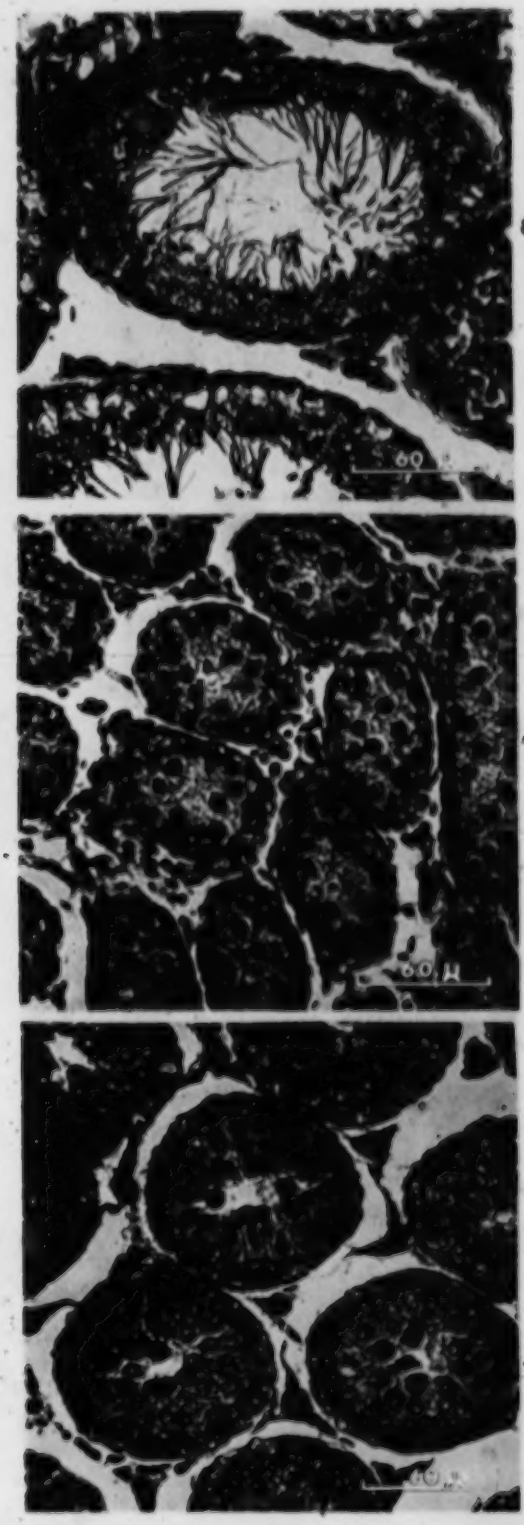


Fig. 20.. Seasonal variations in testes weights of adult male Columbian ground squirrels during the active season. N is presented below each point.

Fig. 21. Cross sections of seminiferous tubules of adult male *S. columbianus* in the following periods: (a) breeding (late April) showing active spermatogenesis, (b) postbreeding (mid-June), showing atrophied condition, and (c) pr hibernation (mid-August) showing late summer recrudescence but no spermatogenesis.


a
60 μ
b
60 μ
c
60 μ

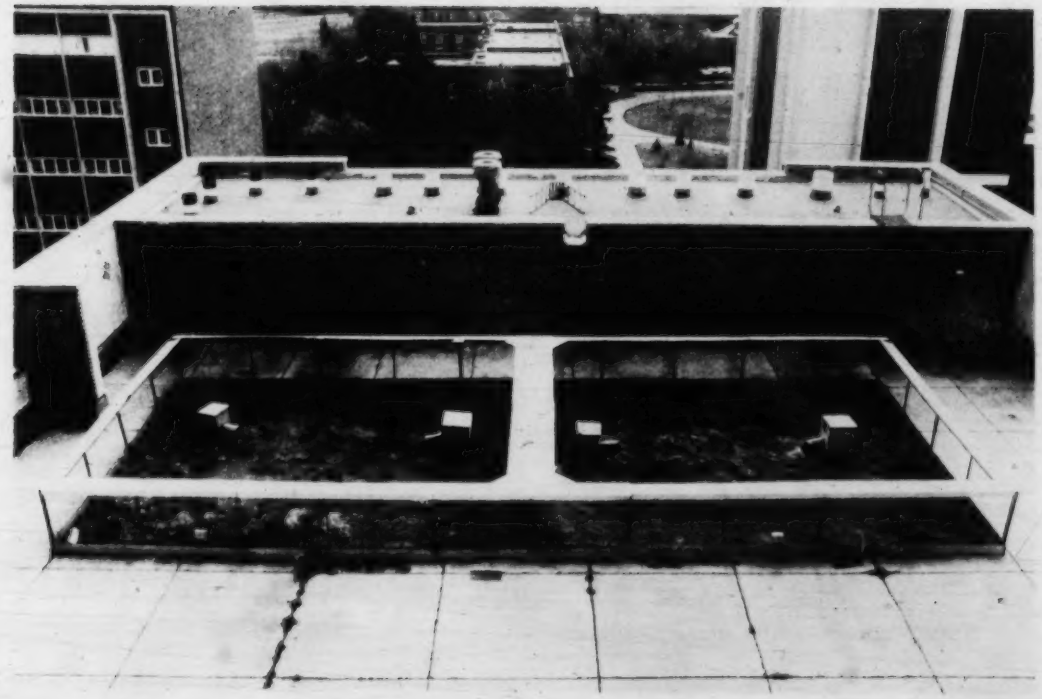


Fig. 22. Outdoor roof pen showing location of divider and animal houses.

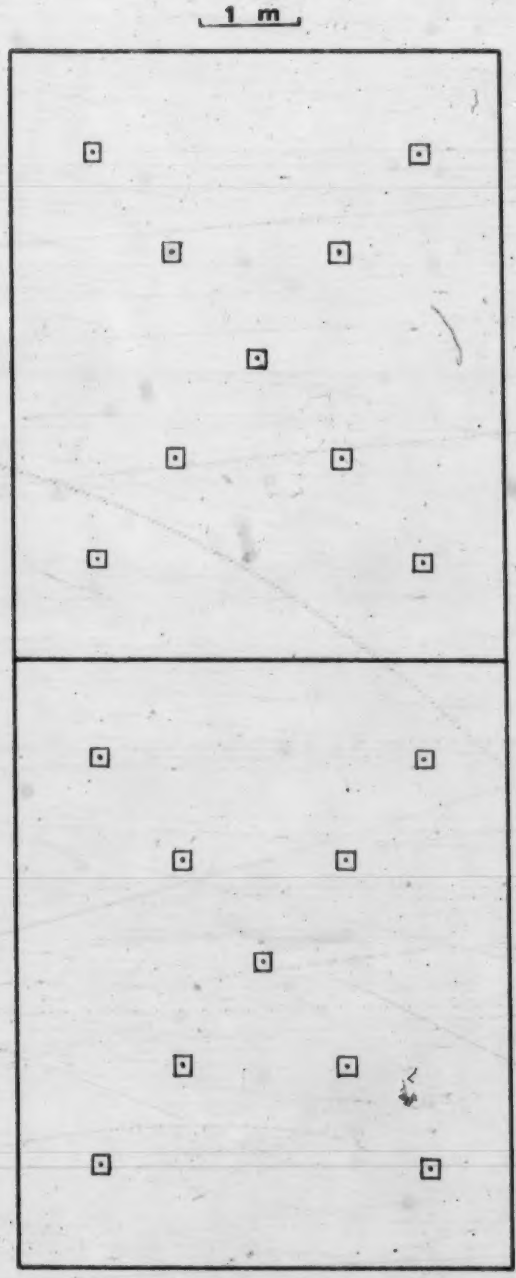
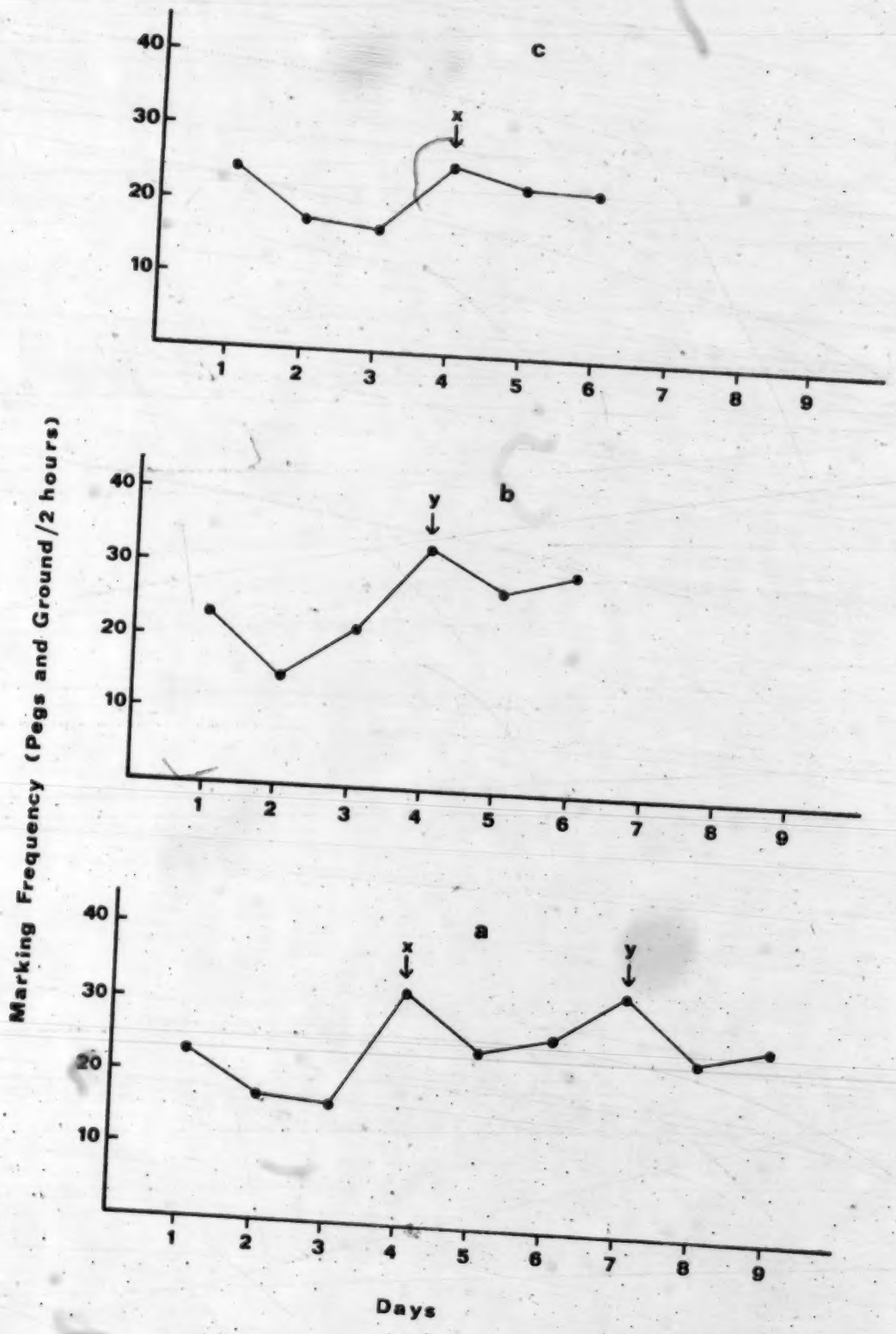


Fig. 23. Diagram of outdoor behavior pen showing the sites where marking pegs were positioned during experimentation.

Fig. 24. Frequency of marking by the resident male in the Scent Marking and Social Setting experiment.

a. = Combination A
b. = Combination B
c. = Combination C
x. = Nonresident male added
y. = Female added


c
b
a
x
y
40
30
20
10
1
2
3
4
5
6
7
8
9
Marking Frequency (Pegs and Ground/2 hours)
Days

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APPENDIX I

Table showing the influences of social setting (Combination A) on frequencies of marking and aggressive encounters for resident and nonresident males plus frequency of clawing for resident males ($\bar{X} \pm S.E._{\bar{X}}$). Each value is a mean for one day.

Day	Marking		Male-Male Aggressive Encounters	Resident Clawing
	Resident	Nonresident		
1	21.9 ± 6.4	-	-	1.8 ± 0.7
2	17.9 ± 4.8	-	-	2.0 ± 1.2
3	17.4 ± 5.0	-	-	1.4 ± 0.6
4	31.6 ± 7.5	0.8 ± 0.5	17.4 ± 4.3	4.1 ± 2.1
5	24.0 ± 6.4	6.1 ± 2.3	10.5 ± 2.4	1.1 ± 0.4
6	26.2 ± 7.7	6.6 ± 1.7	4.8 ± 2.0	1.7 ± 0.6
7	32.0 ± 6.4	5.9 ± 2.7	16.5 ± 4.6	5.4 ± 2.4
8	24.0 ± 4.6	2.6 ± 1.2	11.4 ± 4.5	3.6 ± 1.2
9	27.8 ± 7.1	8.9 ± 6.3	7.1 ± 3.0	3.6 ± 1.1

APPENDIX II

Table showing the influences of social setting (Combination B) on frequencies of marking and clawing by resident males ($\bar{X} \pm$ S.E.). Each value is a mean for one day.

Day	Marking	Clawing
1	23.0 ± 7.2	2.0 ± 1.2
2	14.9 ± 3.5	1.0 ± 0.5
3	21.3 ± 5.8	1.4 ± 0.7
4	32.4 ± 12.3	4.4 ± 2.1
5	27.9 ± 7.1	3.3 ± 1.1
6	30.0 ± 8.7	3.5 ± 1.5

APPENDIX III

Table showing the influences of social setting (Combination C) on frequencies of marking and aggressive encounters for resident and nonresident males plus frequency of clawing for resident males ($\bar{X} \pm$ S.E.). Each value is a mean for one day

Day	Marking		Male-Male Aggressive Encounters	Resident Clawing
	Resident	Nonresident		
1	25.0 ± 5.6	-	-	3.3 ± 1.6
2	18.6 ± 3.2	-	-	2.4 ± 1.0
3	17.4 ± 4.8	-	-	1.6 ± 0.8
4	25.5 ± 5.4	3.5 ± 2.3	17.9 ± 4.1	5.4 ± 2.1
5	23.3 ± 4.5	2.5 ± 0.8	6.0 ± 1.3	2.6 ± 1.2
6	22.6 ± 5.6	11.9 ± 3.9	5.9 ± 0.9	1.9 ± 0.9